The Journal of Laboratory and Clinical Medicine

VOL. XVIII ST. LOUIS, MO., SEPTEMBER, 1933 No. 12

CLINICAL AND EXPERIMENTAL

INSULIN RESISTANCE AND SENSITIVITY*

W. G. KARR, PH.D., C. W. SCULL, PH.D., AND O. H. PETTY,† M.D.
PHILADELPHIA

THE use of insulin in the treatment of diabetes is usually accompanied with little discomfort. However, there occur cases in which there is a more or less reaction to the insulin injected. It is usually of an immunologic nature. Immunologic reactions to insulin may be divided into two classes, one, in which the reaction is local and usually accompanied by swelling, redness, and pain, and the other, in which the reaction is general, and the usual response is urticaria. Urticarial responses were more common with the very crude insulin that was used during the first period of insulin therapy. They were often attributed to the protein content of the insulin. However, with purified insulin, reactions still occur with more or less frequency.

Allan[1] states that the incidence of these local reactions has increased during the past five years. During the year 1930 it occurred in over 13 per cent of the cases at The Mayo Clinic. This reaction was usually mild and was relieved either by change of the type of insulin or passed off spontaneously, as the treatment continued. However, in certain cases the allergic manifestations were more severe and interfered with treatment. Usually these sensitive cases were not accompanied by resistance to insulin, but there were also certain resistant cases, which were not insulin sensitive. These mild local reactions are not uncommon in other clinics, and there is an opinion that they may occur less frequently with insulin prepared from beef than from pig.

*From the Division of Metabolic Diseases of the Philadelphia General Hospital, and the Laboratories of the Graduate School of Medicine of the University of Pennsylvania.
Received for publication, October 10, 1932.
†Deceased.

The following case differs from those previously reported, in that the patient was both very sensitive and very refractory to insulin. Also, the refractory condition disappeared suddenly after twenty-one months and followed immediately the intramuscular injection of some rabbit serum, the nature of which will be described later. The sensitivity to insulin, however, persisted for a much longer period of time and is present, to some extent, after a period of over three years, although the patient has been without insulin therapy for a period of nineteen months.

Similar local reactions have been described in the literature. Glasberg, Somogyi, and Taussig[2] in discussing an insulin case, described the local reaction as follows: "From five to seven hours after the administration of insulin a large area of induration appeared at the site of injection, reaching a diameter of 10 cm. in from twelve to fifteen hours; the area was hot, angry and tender for twelve hours more and then gradually faded. The reaction was identical with several brands of insulin."

The patient of the above authors is very interesting, in that she showed a spontaneous drop in insulin requirements. This is pertinent in relation to the present case. Because of hypoglycemia, the amount of insulin was reduced from 320 units daily to 100 units over a period of six days. Dr. Taussig[3] has kindly given me further information since their report was made. During 1928, two years after the above described reactions, the patient was clinically well on 100 U. daily. In 1929 patient was taking about 70 U. daily. March, 1931, the patient reported that she was without glycosuria on 50 U. of insulin per day. This case differs markedly from the present case in that the refractory period was only of about three months' duration. Also, although there was a rather spontaneous recovery from the refractory condition, still the patient, five years later, is requiring 50 U. of insulin daily. Although these authors examined the serum for precipitins, no reaction could be obtained in any dilutions.

Lawrence[4] in discussing local insulin reactions under his group classed as "delayed reactions" describes them as follows: "In such cases during the first day or two of the injections only the usual stinging results. About the third to fifth day, hot, brawny swellings begin to appear at the site of injection, develop fully in six to twelve hours, and have usually disappeared in twelve to thirty-six hours. They may cause much pain and irritation, and can be partly alleviated by cooling lotions." He states that he has never seen them cause trouble for more than three months. He states that sometimes a change of insulin makes them reappear for a week or two.

Tuft[5] in a paper on insulin sensitiveness, reports a case with a marked generalized urticarial reaction. This patient was sensitive to various brands of insulin tried, and also to purified crystalline insulin. This case gave a marked skin reaction, a positive Prausnitz and Kustner reaction, and a precipitin reaction which remained positive for a short time after the generalized reaction occurred.

This patient apparently was definitely sensitive to purified insulin, but the response was generalized rather than local, as in the present case.

The patient considered in this paper showed, at all times, a marked local reaction at the site of insulin injection. The patient said it came from the very start of injections. It was first noted on the nurse's record ten days later. Soon after injection the patient noted a feeling of warmth in the area. From one to three hours later there appeared a diffuse swelling, very red, which felt much warmer than the remainder of the skin. Pain developed in the area, which persisted for twenty-four hours or longer. This reaction came with every insulin injection and persisted throughout the course of insulin therapy. Later the insulin was injected at various sites over the body in order not to repeat the reaction in the same area too often. In the later few months of insulin therapy, the local reaction changed in some respects. Instead of the diffuse swelling extending for 10 to 15 cm. it became more localized and somewhat indurated. The redness did not appear, instead, a slightly elevated blanching of the skin, a rather typical wheal reaction, extending with a diameter of 4 to 6 cm. about the site of injection. The patient often noted a flushing, or general feeling of warmth all over the body. The pain was present as before. No urticaria was noted until the latter part of March, 1930, when, because of the extremely high insulin requirement, some was given intravenously. When given ten units three times a day there was some urticaria, especially of the lower limbs. This was increased to a 25 U. dosage and after one of these there was a sudden generalized urticaria which was relieved by adrenalin. Reducing this to 15 units three times a day there was little or no reaction noted over a period of time. As mentioned above this intravenous insulin accompanied a larger dose subcutaneously. Later when the insulin requirement dropped the intravenous insulin was stopped. After this at various times there was some urticaria after the regular subcutaneous injections. At one particular time when 90 units were given instead of the customary 45, there was a sudden marked generalized urticaria which was relieved by adrenalin.

Root[6] in an excellent review has summarized some thirteen conditions which might influence the resistance of the patient to insulin. Throughout the course of this patient's stay in the hospital various clinical conditions were looked for, which might, in some way, aid in explaining the refractory reaction to insulin. Various liver functional tests were instituted, but they were all negative for any hepatic dysfunction. Basal metabolic rate determinations done during August, 1929, gave readings of about + 24. There was no evidence of hyperthyroidism, however, and later basal metabolic rates were normal. Outside of a few common colds and an abscess of the thigh there were no known infections present. There was a cardiac condition, but it was not accompanied by either circulatory failure or edema, which might affect the absorption of the insulin.

Of the theories advanced for insulin resistance, it would seem that only the one concerned with an enzyme of normal muscle which reacts with glucose only in the presence of insulin might be applicable. It is interesting to speculate concerning the relationship between the sensitivity to insulin and the refractory condition shown by the patient. Insulin sensitive cases are somewhat common. True insulin refractory cases are rather rare. It is noted in the

case of Glasberg, Somogyi, and Taussig[2] that while their case was both refractory and sensitive they did not parallel each other. In the present case sensitivity has been present throughout the refractory period and also although the patient has been without insulin for a number of months, it is still present. With the development of general allergic symptoms, and with more marked skin reactions, it would seem that the patient became even more sensitive to insulin in the later course of insulin therapy than she was in the middle of the therapy when the tests were first instituted. At present, as discussed later, the patient has lost much of the sensitivity.

The following is a summary of the Clinical Course of the patient:

Patient C. E. was a white woman, aged fifty-eight. She was admitted to the dermatologic wards of the Philadelphia General Hospital because of pediculosis corporis and a shallow varicose ulcer over the middle of the right tibia. In addition she had moderate edema of both ankles which she had first noted about four months previously. Also she had been dyspneic on exertion for about six months previously.

Her past history revealed that she had been in comparatively good health in her early years. In January, 1922, she had her gallbladder removed and nine months later her uterus. Menopause had occurred at forty-three.

On admission her temperature, pulse, and respiration were normal, and the patient was not acutely ill. She had many scratch marks on her back, shoulders, and arms, a shallow ulcer of two inches in diameter about the middle of the right tibia, there was moderate edema of both ankles, a scar in the right upper quadrant of the abdomen, and a midline suprapubic scar.

Examination of the heart and lungs showed no gross pathology. Her blood pressure was 145/75; red cell count, 4,490,000; white cell count, 12,200; urine showed no albumin, sugar, or casts; blood urea nitrogen, 11 mg.

No symptoms of diabetes had been elicited but her fasting blood sugar was 197 mg. and on Dec. 21, 1928, the patient was transferred to the Metabolic Division.

After her admission to the Metabolic Division, the patient was subject to frequent attacks of precordial pain of anginoid character, which were relieved by vasodilators. These attacks were frequent, at times more than once daily and continued for over a year. On several occasions there was an associated pulmonary congestion. Beginning about April, 1930, they became less and less frequent and she has had none since July 1, 1930.

Electrocardiographic tracings taken at various intervals always showed evidence of left ventricular preponderance and several times frequent extrasystoles.

X-ray examination of the chest showed nothing of importance except some spondylitic changes in the lower dorsal vertebras.

There were no foci of infection found and the varicose ulcer healed in a few weeks.

On several occasions the urine showed many leucocytes but this finding did not persist and there was no other evidence of genitourinary infection. The urine at no time showed casts, the phenolsulphonephthalein excretion was normal and there was no nitrogen retention in the blood.

The temperature was normal throughout the period of hospitalization with the exception of a few occasions when slight rises, not above 101° F. occurred for twenty-four to forty-eight hours. These transient elevations of temperature were coincident with and apparently the results of minor ailments, such as diarrhea, bronchitis, etc.

A normal bilirubin content of the blood plasma, no retention bromsulphalein dye at the end of one-half hour and no increase of urobilinogen in the urine, indicate no liver dysfunction.

The red blood cell count varied between 5,050,000 and 3,250,000 during the period in the hospital. When discharged the count was 3,250,000. Later, on Aug. 4, 1931, the red blood cell count was 3,970,000.

The white count since admission to the Metabolic Division had varied between 5,000 and 9,700. The differential counts were normal.

Fig. 1 shows a graph of the insulin requirement of the patient made by taking approximate monthly averages of insulin dosage. The decrease after the eighth month followed a change in the manner of giving the insulin.

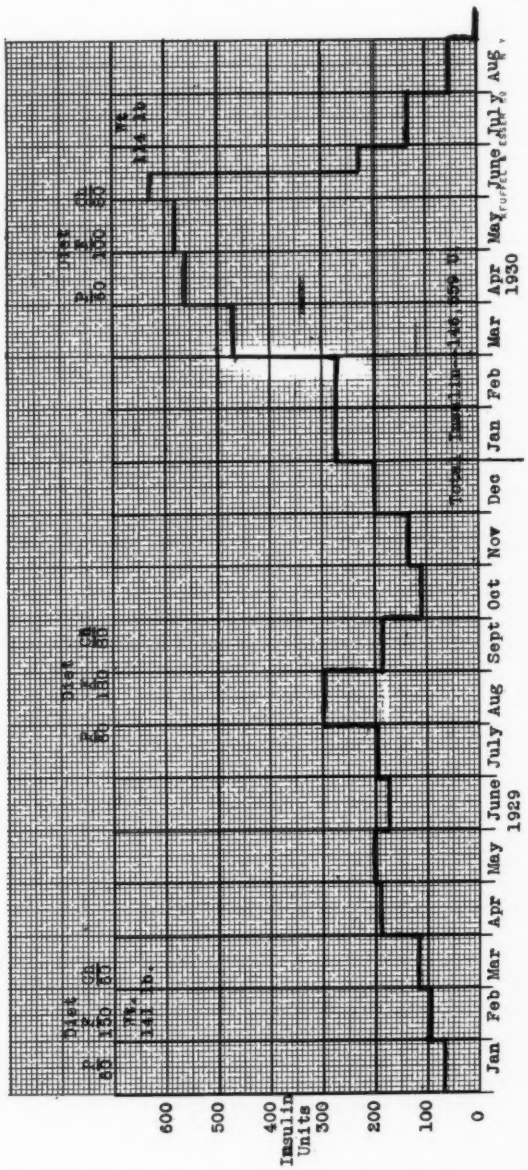


Fig. 1.

From this time on it was given at various sites over the body rather than just in the arms. This procedure may have made it more effective for a time.

During the month of March, 1930, when the refractory condition of the patient was becoming more marked, certain tests were instituted. One-tenth cubic centimeter of Lilly's U. 20 pig insulin was given intradermally and a

definite wheal reaction was obtained. A supply of beef insulin was obtained and a similar sensitivity was shown. Through the kindness of Dr. E. M. K. Geiling, of Dr. Abel's laboratory, a supply of crystalline insulin was obtained. This gave the same typical skin reaction as the commercial insulin. A quantity of this crystalline insulin was dissolved sufficient for two therapeutic doses, which was given to the patient with the same local reactions as the commercial insulin. The cutaneous reaction was positive to tests repeated at intervals while the patient was in the hospital, and when tested some time after the rabbit serum had been given seemed to give even a greater reaction than formerly. For a period of about one year no cutaneous reactions were tried. In April, 1932, when tested cutaneously to Lilly's U. 20 insulin, a beef insulin, and crystalline insulin, she only gave a positive reaction to the Lilly's U. 20 insulin. While this was definitely positive she is no longer sensitive to crystalline insulin, and it would seem that her sensitivity is gradually disappearing. Why she was originally sensitive to crystalline insulin and now only to a commercial insulin is an interesting speculation.

In view of the large insulin requirement during March, 1930, it was thought that there must be some combination of the insulin in the patient's body. An attempt was made to ascertain whether this combination could be shown in vitro. The patient's serum was mixed with a quantity of insulin just sufficient to produce convulsions in a rabbit, and as a control normal serum was mixed in a like manner. These mixtures were given to various rabbits, and as far as these brief experiments could demonstrate there was no combination of the insulin with the patient's serum. Blood sugar lowering in the rabbits was just as great with the mixture of the patient's serum as with the mixture with normal serum. Three serums were procured at this time, one from a normal individual, one from a diabetic, and one from the patient. These three unlabeled serums were sent to the laboratory to be tested for precipitins. U. 20 insulin was used as an antigen and the various serums were diluted. It was possible to demonstrate precipitins in the patient's blood, and it was identified in this manner from the other two serums. The results are given in Table I.

TABLE I

SERUM DILUTIONS

SERUM	10	20	40	80	160	320	640	CONTROL
A—(Normal)	–	–	–	–	–	–	–	–
B—(Patient)	++	++	++	++	–	–	–	–
C—(Diabetic)	–	–	–	–	–	–	–	–

As a further study for allergic antibodies the Prausnitz and Kustner passive transfer was tried. A small amount of the patient's serum was introduced intradermally into a normal person's arm. Twenty-four hours later an intracutaneous injection of a small amount of insulin was made in the same area as well as at another point, which was not sensitized. A wheal developed at the site of insulin injection in the sensitized area, while similar injection at another site was negative. This test was repeated again in April, 1932, and found to be negative.

With the thought in mind that there still was some immunologic combination of insulin with the patient's serum, a rabbit was sensitized to a mixture of 10 c.c. of the patient's serum and 2.5 units of insulin. This was done with graduated doses over a period of about three weeks. Continued attempts were made to test the patient's blood as well as this particular sensitized rabbit blood for precipitins. Insulin was used as the antigen and various dilutions of the serum were added. Although the one series of precipitin tests reported above seemed rather definite, difficulties were encountered in further trials with both the rabbit's and the patient's serum. Flocculation even occurred in the control tube and a prezone developed which later was shown to depend on the P_H of the medium. In view of these difficulties it seems desirable not to report what were first thought to be definite precipitin reactions. However, in view of the fact that there was a possibility of certain antibodies in the sensitized rabbit serum, some of this serum was given to the patient. The patient was found sensitive to the rabbit serum and was desensitized by gradually increasing doses, until finally 5 c.c. of the rabbit serum was injected intramuscularly. At this particular time it was necessary to give the patient over 600 U. of insulin and with that dosage blood sugars were not kept under 200 mg. To make sure that the patient actually required a large amount of insulin, it was markedly reduced one day and at another time cut completely for twenty-four hours. The patient responded with a blood sugar of 320 mg., CO_2 25, and a blood sugar of 328 mg., CO_2 23 respectively. The blood sugars were not especially high, but the acidosis was rather alarming.

About six days after the rabbit serum was given, the patient developed insulin shocks in a very surprising manner. The insulin was very rapidly cut during the next two to three days, with other insulin shocks occurring. Within a period of one to two weeks the patient's blood sugar was well controlled with from 100 to 150 U. of insulin daily. After about one month at this level the insulin requirement dropped further and in October, 1930, the insulin therapy was discontinued. After insulin was totally withdrawn from the patient, blood sugars were about 90 mg. The diet, therefore, was increased to contain 300 gm. of carbohydrate daily. This increase was gradual and was made during a period of about five weeks. During that period the blood sugars averaged 85 mg. A few days later, November, 1930, the patient was discharged to the Out-patient dispensary and told to go on an unlimited carbohydrate intake. The dispensary blood sugars, on this regimen, averaged 115 mg. In December it was decided to do a glucose tolerance test, as the means of checking her diabetic condition. The following data were obtained:

	Fasting	½ hr.	1 hr.	2 hr.	3 hr.
Blood sugar, mg.	85	180	284	320	200
Urine sugar, per cent	0	–	0.6	1.6	1.0

It was obvious that the patient was still diabetic. Dispensary blood sugars for the month following the tolerance test averaged 125. The patient gained five pounds during this period.

In view of the sugar tolerance and the rather high fasting blood sugars, patient was again put on a prescription diet, containing 150 gm. of carbo-

hydrate. The dispensary blood sugars for the following two months averaged 126 mg. with one of them 140 mg. In view of these continued slightly elevated fasting blood sugars, the carbohydrate was cut to 120 gm., which diet she has remained on for the past fourteen months, until the present time. This diet contains 2050 calories, and has maintained her weight rather uniformly at about 116 pounds.

Fasting blood sugars over this period were usually taken at a three-week interval, and have averaged 124 mg. One of these was 159 mg. which immediately followed an attack of mild influenza. None of these sugars during this period have been under 108 mg. It would seem as if there was a tendency toward the aggravation of her diabetic condition. She is an intelligent person, and we believe, tends more or less faithfully to follow her prescription diet. It should be noted that during her stay in the hospital a gastric analysis showed no free hydrochloric acid present. She was put upon hydrochloric acid therapy and on complaint of gastric distress during her visit to the dispensary another gastric analysis was made. This showed a slight hyperchlorhydria. The acid was discontinued.

DISCUSSION

There are many interesting speculations concerning this case, with very little information as to exactly what factors were concerned in producing the interesting changes noted.

The patient was very obviously sensitive to insulin itself, as far as we could determine, and not to any contaminating products of a protein nature. It is generally conceded that a total diabetic would probably not need more than 100 to 200 U. of insulin, under conditions where no coma or infection was present. In this case, as in other cases, attempts were made to find insulin in the urine, and this was unsuccessful. It is quite obvious then that the insulin was either combined or destroyed in the body. The tests indicate that the combination might have been of an immunologic nature. It would seem to be more probable that it is a combination of this nature rather than of a lack of some substance in the tissues, such as a muscle enzyme.

The sudden drop of insulin requirement might have been entirely spontaneous, but it is difficult to explain its occurring so soon after the injection of rabbit serum, if this were the case. It is, of course, possible that a nonspecific protein reaction produced some reaction which was responsible for this sudden drop in insulin requirement. It is also possible that the rabbit serum contained some antibodies which might have combined with the substance in the patient which had previously combined with the insulin. It is, however, most difficult to speculate at all concerning the nature of such antibodies. Therefore, it seems advisable to consider the case as one in which the refractory condition with regard to insulin was suddenly abated. The added information may only induce certain considerations with regard to its cause. The patient still remains sensitive to pig insulin, and is still a mild diabetic, which condition, at the present time, can be controlled by diet without insulin therapy.

SUMMARY

A case is presented of a patient who was both very sensitive and very refractory to insulin therapy. The refractory condition has disappeared, while the sensitivity persists to some extent eighteen months after withdrawal of insulin.

The patient at one time required over 600 U. of insulin daily to prevent acidosis, although the fasting blood sugars were all over 200 mg. The patient has been without insulin for eighteen months, and although still diabetic is well controlled by a prescription diet.

The refractory condition of the patient disappeared very suddenly about one week after the intramuscular injection of a rabbit serum, which may have contained antibodies, as the animal was sensitized to the patient's serum plus insulin.

We wish to express our thanks to Dr. William Kreidler, for doing the serologic work, and to Dr. Louis Tuft, for advice and aid in performing some of the immunologic reactions.

REFERENCES

1. Allen, F. N.: Personal Communication.
2. Glassberg, Somogyi and Taussig: Diabetes Mellitus. Report of a Case Refractory to Insulin, Arch. Int. Med. **40**: 676, 1927.
3. Taussig, A. E.: Personal Communication.
4. Lawrence: Local Insulin Reactions, Lancet **1**: 1125, 1925.
5. Tuft: Insulin Hypersensitiveness: Immunologic Considerations and Case Reports, Am. J. M. Sc. **176**: 707, 1928.
6. Root: Insulin Resistance and Bronze Diabetes, New England J. Med. **201**: 201, 1921.

GLUCOSE TOLERANCE CURVES IN PULMONARY TUBERCULOSIS*

Observations Upon One Hundred Cases

David W. Kramer, M.D., Philadelphia, Pa.
With the Technical Assistance of Freda Ward

THE relationship between tuberculosis and diabetes is by no means clear. The existence of the two diseases in an individual is not so uncommon as one might believe. When this combination is found, the history of the diabetic condition usually preceedes the onset of the tuberculosis. It is the exceptional case in which conditions are reversed, i.e., the development of diabetes mellitus in an individual who already has tuberculosis. Why the rarity? Is it possible that in tuberculosis there is present an overactivity or hyperfunctioning of the islands of Langerhans? This would no doubt give these patients a higher tolerance for carbohydrates and a lessened tendency to diabetes. Lundberg[1] seems to have taken this attitude. On the other hand, Langston[2] found that 20 of 29 patients (60 per cent) showed a Type I curve, i.e., a high blood sugar curve indicating diminished tolerance for carbohydrates. Rohdenberg, Bernhard and Kriebel[3] in their studies also reported that a Type I blood sugar curve was present in 60 per cent of their patients with tuberculosis. John[4] in his study commented on the high incidence of diabetic curves in tuberculous individuals. MacLean and Sullivan[5] found a normal tolerance for sugar in two children who had generalized tuberculosis.

The difference of opinion and the divergent blood sugar curves seem to indicate that the problem is a complex one. Considering the fact that diabetes is a rare development in tuberculosis, we were impressed with the fact that glycosuria, on the other hand, is not uncommonly found. The blood sugar in these patients as a rule is within normal limits. In such cases, the first impression would suggest an alimentary or renal glycosuria.

We felt that further studies were warranted and hoped that a study of the blood sugar curves after the ingestion of glucose may throw some light upon the problems mentioned above.

One hundred patients suffering from pulmonary tuberculosis in various stages of the disease were selected at the Eagleville Sanatorium for the study. Two series were observed. One group (Series A) consisted of 50 patients who at one time or other showed the presence of glycosuria. Definitely established cases of diabetes were not considered. The second group (Series B) consisted of patients who never showed sugar in the urine. In other respects, the cases were unselected.

*From the Department of Clinical Research, Eagleville Sanatorium.
Received for publication, October 17, 1932.

The method of study and the interpretation of the blood sugar curves deserve some comment. After withdrawing blood, on a fasting stomach, the patient was given 1.75 mg. glucose per kilogram body weight. Samples of blood were subsequently taken from the vein thirty minutes, one and two hours after the ingestion of the sugar. The technic advocated by Folin and Wu[12] was followed in making the blood sugar estimations.

Specimens of urine were collected before and two hours after the test for analysis.

The proper interpretation of the blood sugar curves was a problem. Unfortunately, there is no uniform classification. Suggestions for grouping the various curves have been offered by Langston, John, Hale-White and Payne,[6] and Mosenthal.[7] The types mentioned by Langston seem to be the most acceptable.

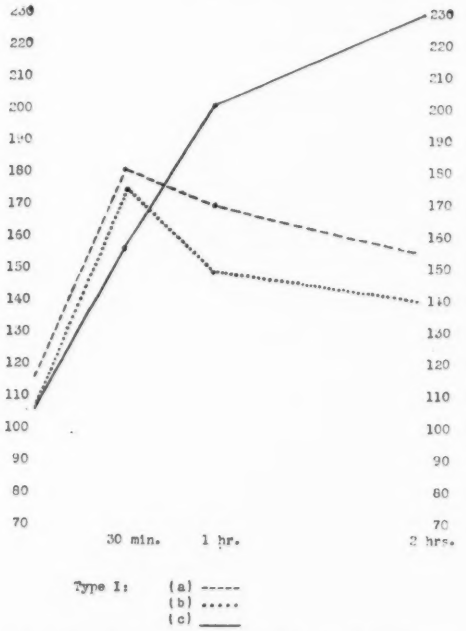


Fig. 1.—Showing Type I, the high or diabetic curve.

He refers to the curves which reach 180 mg. or above and fail to return to normal levels at the end of the two hours as Type I. The normal curve, with its fasting level between 90 and 120 mg., rises to 160 and, at the end of two hours, returns to approximately the original figure, as Type II. "Type III begins within normal limits, does not rise above the initial figure, and at the end of the test period is as low or lower than in the beginning. This is the type of curve we should expect to find in hypoactivity of the thyroid, suprarenals and pituitary glands."

After collecting 100 blood sugar curves in our studies of tuberculous patients, we had some difficulty in placing them in their proper categories. Accepting Types I, II, and III suggested by Langston, subgroups were arranged so as to permit the different curves with variations to be classified more readily.

In Type I, emphasis is made not only upon the height of the rise but also the level of the blood sugar at the end of the two-hour period. This is stressed

by Brill[8] and Kilduffe[9] and seems to conform with the consensus of opinion today. Type I can be divided into three groups: a, b, and c. In subgroup (a) the blood sugar rises to 170 or above the accepted "spilling point" 180 mg. within thirty to forty-five minutes and usually remains above this level at the end of the two-hour period; (b) the initial rise is usually between 160 and 170 mg. and may drop slightly but does not return to normal limits at the end of the test; (c) the blood sugar curve has a tendency to rise steadily, continues upward and fails to drop during the period of observation. The subgroups are plotted in Fig. 1.

In Type II which embraces the normal groups, the fasting blood sugar conforms to the normal limits from 90 to 120 mg. After the ingestion of glucose there is a rise at the end of thirty to forty-five minutes to about 160 mg.,

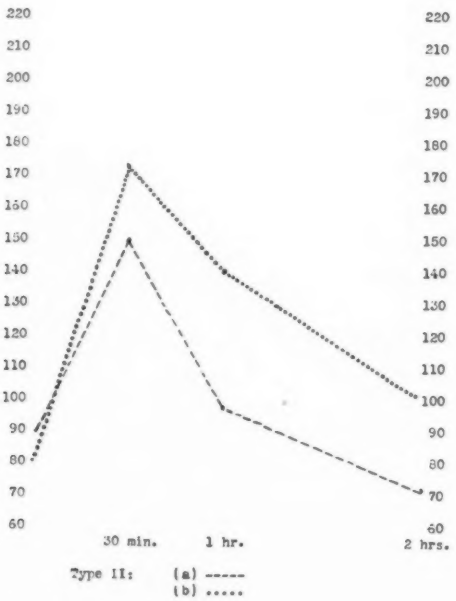


Fig. 2.—Showing Type II, the normal curve.

the blood curve then returns to the original level or slightly below at the end of the two hours. This group may be termed as Type II-a. The blood sugar in some cases rises to 170 mg. or even higher, but gradually drops so that at the end of the test, the curve reaches normal limits. This group is labeled Type II-b (Fig. 2).

In Type III we are dealing with curves of low range. Some are inclined to include them in the normal group. However, the mere fact that they are not diabetic curves does not necessarily mean that they are normal. When a definite amount of glucose is ingested it is presumed that a rise of blood sugar will result even if it is only temporary. Should this fail to occur then some explanation should be sought to explain this reaction which is not in line with the normal curves described above. Thaysen and Norgaard[10] define the low curve as one

characterized by the small height of its rise, approximately 40 mg. per hundred c.c. or less. It is also understood that the fasting blood sugar is at the lower normal level or below. However, in the classification offered in this paper, it is suggested that we include those who may have a fasting level of 120 or 130 mg. but do not rise above this; on the contrary there is a drop to lower levels after the ingestion of glucose, as evidenced by our subgroup (b). Type III therefore may be divided into three subgroups: (a) in which there is a slight rise, less than 40 mg. and then a drop to lower levels; (b) in which the fasting blood sugar is within normal range and drops progressively down to subnormal levels and (c) in which the sugar curve drops below the fasting level and later rises at the end of the two-hour period but the rise is not more than 40 mg. above the initial fasting level (Fig. 3).

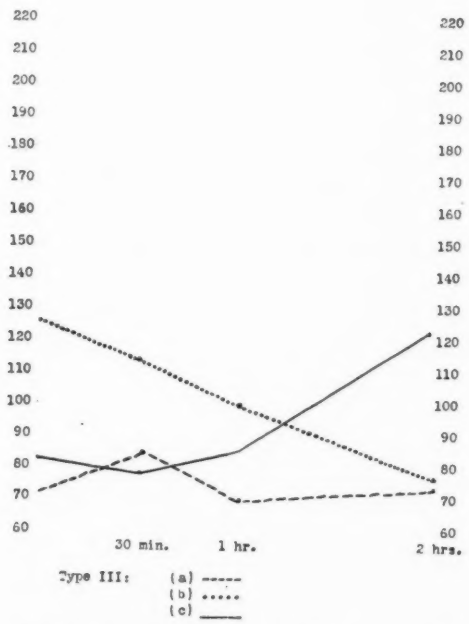


Fig. 3.—Showing Type III, the low curve.

The blood curves were classified according to the groups or types suggested in Figs. 1, 2, and 3. Incidentally, it may be mentioned that the subgroups of the various types were selected from the sugar curves of the patients studied in the course of our observations. The following results were obtained: in Series A, there were 12 cases (24 per cent) showing Type I which may be referred to as the diabetic curve; 28 cases (56 per cent) with a normal curve or Type II; and 10 cases (20 per cent) with a low curve, Type III. Collectively, there were 38 patients (Types II and III) who had nondiabetic curves.

In Series B, the control group, there was a tendency to higher percentages of the nondiabetic curves, viz., 31 cases (62 per cent) with Type II and 14 cases (28 per cent) with Type III. Collectively, 45 patients out of 50 in this series had nondiabetic curves giving a percentage of 90 in comparison with the 76

TABLE I

SHOWING NUMBERS AND PERCENTAGES OF THE DIFFERENT GROUPS IN SERIES A AND B

	SERIES A (GLYCOSURIA GROUP)		SERIES B (AGLYCOSURICS)		A AND B TOTAL
Type I Diabetic	a – 7 cases b – 2 cases c – 2 cases	12 cases (24%)	a – 1 case b – 1 case c – 3 cases	5 cases (10%)	17
Type II Normal	a – 20 cases b – 8 cases	28 cases (56%)	a – 17 cases b – 14 cases	31 cases (62%)	59
Type III Low	a – 8 cases b – – c – 2 cases	10 cases (20%)	a – 12 cases b – 1 case c – 1 case	14 cases (28%)	24

per cent in Series A. Originally our object in studying a group of controls was merely to increase the number of cases for observation. There was little to choose between the patients in both series so far as the tuberculosis was concerned. The fasting blood sugars were similar; age, sex, and basal metabolic studies varied but slightly in the two groups. The deciding factor in their classification was the presence or absence of glycosuria. In the majority of the cases, sugar was found only once. Despite this fine distinction between Series A and B, there is a higher percentage (24 per cent) of the diabetic curve and a much lower percentage of the nondiabetic curves (76 per cent in comparison with the 90 per cent) in Series A, the glycosuria group.

Another point of interest is the tendency for the patients in Series A to "spill over" when the glucose tolerance test was performed. A glance at Table II shows 33 patients of Series A had glycosuria at the end of the two-hour period, a percentage of 66 in comparison with the 18 cases (36 per cent) of Series B.

For convenience, an analysis of the 100 cases was made in reference to age, sex, family history of diabetes, history of glycosuria, and basal metabolism. This information is arranged in Table II.

The total number of diabetic curves in both series was 17 or 17 per cent. This is much lower than the figures of Langston and Rohdenberg, et al. Langston reported 60 per cent high curves in tuberculosis with a slightly lower percentage (50 per cent) in incipient cases. Rohdenberg also mentioned finding Type I in 60 per cent of tuberculous patients.

When one considers that we may expect to find a certain percentage of high curves even in groups of apparently normal individuals and add to this the fact that in the presence of infections there is a tendency to a lessened tolerance for carbohydrates, the findings of 17 per cent high curves in tuberculosis does not seem unduly excessive.

The other side of the problem is: does tuberculosis in some way bring about an increased tolerance for carbohydrates? It is impossible to answer this question without becoming involved in lengthy discussions which are not intended in this paper. Our object in making this study in tuberculosis was to observe how these patients would react to blood glucose tolerance tests and interpret our findings accordingly.

TABLE II

SHOWING AGE, SEX, URINE FINDINGS AND BASAL METABOLIC STUDIES OF THE VARIOUS GROUPS

SERIES A

SERIES B

		TYPE I A	TYPE I B	TYPE I C	TYPE II A	TYPE II B	TYPE III A	TYPE III B	TYPE III C	TOTAL A	TOTAL	TYPE I A	TYPE I B	TYPE I C	TYPE II A	TYPE II B	TYPE III A	TYPE III B	TYPE III C	TOTAL B	TOTAL A AND B
Age:	11 – 20	1	–	2	3	–	1	–	–	7		1	1	–	5	3	3	1	–	14	21
	21 – 30	1	1	–	11	4	7	–	2	26		–	–	1	8	6	8	–	1	24	50
	31 – 40	1	1	–	2	–	–	–	–	4		–	–	1	–	4	1	–	–	6	10
	41 – 50	3	–	–	3	2	–	–	–	8		–	–	1	3	2	–	–	–	6	14
	51 – 60	1	–	1	1	2	+	–	–	5		–	–	–	–	–	–	–	–	0	5
		7	2	3	20	8	8	–	2			1	1	3	16	15	12	1	1		
			12			28		10		50			5			31		14		50	100
	Total																				
Sex:	Male	4	1	–	10	5	1	–	–	21		1	1	1	10	8	4	1	–	26	47
	Female	3	1	3	10	3	7	–	2	29		–	–	2	6	7	8	–	1	24	53
Family history. Diabetes		–	–	1	2	–	–	–	–	3		–	–	1	–	1	–	–	–	2	5
Urine after test:	Negative	1	–	–	7	3	4	–	1	16		–	–	1	12	7	7	1	1	29	45
	Pos. 2 hr.	6	2	2	13	5	4	–	1	33		1	1	2	3	6	5	–	–	18	51
	Not mentioned	–	–	1	–	–	–	–	–	1		–	–	–	1	2	–	–	–	3	4
Glycosuria		4	2	2	7	6	4	–	3	28		–	–	–	–	–	–	–	–	0	28
Number of times:	1	2	–	–	1	1	3	–	–	6		–	–	–	–	–	–	–	–	0	6
	2	–	–	–	1	1	–	–	–	2		–	–	–	–	–	–	–	–	0	2
	3	–	–	–	–	–	–	–	1	4		–	–	–	–	–	–	–	–	0	4
	4 or more	2	–	1																	
Basal Metabolism	+31 to +40	–	–	–	1	–	–	–	–	1		–	–	–	–	–	–	–	–	0	1
	+21 to +30	–	–	–	1	1	–	–	–	2		–	–	–	–	–	–	–	–	0	2
	+16 to +20	–	–	–	–	–	1	–	–	1		–	–	–	1	1	–	–	–	2	3
	+11 to +15	–	1	–	2	1	1	–	–	5		–	–	–	3	1	–	–	–	7	12
	+ 1 to +10	–	–	–	5	2	3	–	1	11		–	–	–	3	3	3	–	–	8	19
	–0–	–	–	–	1	–	2	–	–	3		–	–	–	–	2	–	–	–	2	5
	– 1 to –10	2	–	1	2	1	1	–	–	7		1	–	–	3	3	3	–	–	10	17
	–11 to –15	–	–	1	–	–	–	–	–	1		–	–	–	–	–	–	–	–	0	1

Low blood sugar curves occur in 5 per cent of normals according to the studies of Thaysen and Norgaard. They commented upon the dearth of literature upon this subject and reported the frequency of low blood curves in sprue and Gee-Herter's disease: 15 of 30 patients (50 per cent) presenting this finding. In our series of tuberculous patients, the presence of the low curve in 24 per cent seems to be significant. The explanation of this is not a simple matter and various theories may be offered. Is it due to a failure of the intestinal tract to digest the sugar, is it one of the manifestations of hypofunction of the endocrine glands or is there a hyperfunction of the islands of Langerhans? In Thaysen and Norgaard's studies on steatorrhea, they were convinced that the sugar was utilized and that the low curves could not be explained on these lines. However, their experiences were not based on tuberculous patients. Shay[11] noticed that in patients who had evidences of ileocecal tuberculosis, there was a failure to fully digest carbohydrates, but tuberculous patients without this intestinal complication showed complete digestion of the sugars. However, there were no cases of ileocecal tuberculosis found in this group.

It has been suggested that the low curves may be due to a hypofunction of the ductless glands. In our observations the patients showed no evidences of endocrinopathies such as myxedema or hypopituitarism. Although basal metabolic studies were not performed on all of the patients, yet we were fortunate in having some records made. Basal metabolic studies were performed on 17 of the 24 patients in Type III with the following results: 6 cases were between +1 to +10; 4 cases were between +11 to +15; 1 case was between +16 to +20; 2 cases were 0; 4 cases were between –1 to –10.

Since we cannot explain the increased frequency of this curve by a deficiency in the consumption of sugar or hypofunction of the endocrines, may it not be construed as an argument in favor of tuberculosis having some influence on the carbohydrate tolerance? Where or how this influence is exerted we are not prepared to say at this time.

The question was raised as to whether or not tuberculosis has any effect upon the sugar tolerance curve. The patients were analyzed according to the type of tuberculosis, the state of their general condition and a comparison was made with the various blood sugar curves that they presented. There were 16 incipient cases in the two series. None of the incipient cases had diabetic curves; 10 showed normal curves and 6 had low curves. Of the 64 moderately advanced cases, 13 showed high blood sugar curves of the diabetic type, 35 were in the normal group and 16 had low curves. There were 18 patients who had a fairly advanced type of tuberculosis, 4 showed diabetic curves, 12 were normal, and 2 were low.

It may be of interest to note that those patients with the milder form of tuberculosis had no high blood sugar curves. Whether this means anything or not it is difficult to say. I doubt whether too much significance may be given to this, because if so, we would expect the larger number of high curves in those patients with the most advanced forms of tuberculosis, which is not true.

As to the type of curve found in comparison with the patient's condition: of the 65 patients who were improved, 7 showed diabetic curves, 39 had normal curves, and 18 had low curves. Of the 26 cases of patients whose condition was

stationary, 5 had high curves, 17 had normal curves, and 6 were low. Of the 5 retrogressing cases, 3 had high or diabetic curves and two were normal curves. Although the retrogressing cases showed higher percentages in the diabetic curves, the number is so small that I doubt whether any importance can be attached to it.

In summarizing, it appears that the incipient cases are less apt to show high curves but this may be due to the fact that this group consisted of younger individuals. The moderately advanced groups showed a slightly higher percentage of diabetic curves than the far advanced. Of the low curves, the percentage of the incipients was highest.

SUMMARY AND CONCLUSIONS

Blood sugar curves were studied in 100 tuberculous patients. These patients were unselected so far as the pulmonary disease was concerned.

They were divided in two groups or series. Series A included those patients who at one time or other had glycosuria. The fasting blood sugars were invariably of normal range. Diabetics were not included in this series. The control group was labeled Series B.

Suggestions of classifying the blood sugar curves are offered. They may be grouped under three main types: Type I, the high or diabetic curve; Type II, the normal curve; and Type III, the low curve.

There were 17 cases of the 100 studied showing a high or diabetic curve. Twelve of this number were found in Series A. Eighty-three cases had non-diabetic curves. Fifty-nine were normal and 24 showed low curves.

The 17 per cent diabetic curves were much below the figures mentioned by some authors.

The 24 per cent low curves impressed us as being significant.

The influence of the state of the tuberculosis upon the blood sugar curve is indefinite. Although from our experience an occasional case of increased tolerance for carbohydrates may be observed in the late stages of tuberculosis, we were unable to point out any direct bearing upon the blood sugar curves in the patients studied.

While the number of cases studied is not large to draw definite conclusions, it would seem that the low percentage of diabetic curves and the comparative high percentage of low curves may be interpreted as an indication that tuberculous patients have a higher tolerance for sugar and may explain, in a way, the rarity of diabetes developing in tuberculosis.

Grateful acknowledgment is expressed to Dr. A. J. Cohen, Medical Director of Eagleville Sanatorium, for his suggestions, courtesies, and cooperation.

REFERENCES

1. Lundberg, E.: Carbohydrate Metabolism in Pulmonary Tuberculosis, Acta Med. Scandinav. **71:** 493, 1929.
2. Langston, W.: Glucose Tolerance Test, J. LAB. & CLIN. MED. **7:** 292, 1922.
3. Rohdenberg, G. L., Bernhard, A., and Kriebel, O.: Quoted by Kilduffe, Clinical Interpretations of Blood Examinations, Philadelphia, 1931, p. 542.
4. John, H. J.: Summary of Findings in 1100 Estimations, Endocrinology **13:** 388, 1929.
5. MacLean, A. B., and Sullivan, R. C.: Dextrose Tolerance in Infants and Young Children, Am. J. Dis. Child. **37:** 1146, 1929.

6. Hale-White, R., and Payne, W. W.: Dextrose Tolerance; Diagnostic Value of Dextrose Tolerance Curves, Brit. Quart. J. Med. **20**: 457, 1927.
7. Mosenthal, H. O.: Interpretation of Sugar Tolerance Tests, Med. Clin. N. Am. **9**: 549, 1925.
8. Brill, I. C.: Effect of Normal Meal Upon the Blood Sugar Level in Health and Conditions of Disease, J. Lab. & Clin. Med. **8**: 727, 1923.
9. Kilduffe, R. A.: Clinical Interpretations of Blood Examinations, Philadelphia, 1931, 532.
10. Thaysen, T. E. H., and Norgaard, A.: Regulating of Blood Sugar in Idiopathic Steatorrhea, Arch. Int. Med. **44**: 22, 1929.
11. Shay, H.: Personal communication.
12. Folin, O., and Wu, H.: System of Blood Analysis; Simplified and Approved Method for Determination of Sugar, J. Biol. Chem. **41**: 367, 1920.

CHOLELITHIASIS

A Statistical Study With Special Reference to Its Frequency in the Colored Race

R. H. Jaffé, M.D., Chicago, Ill.

NUMEROUS statistical studies have been devoted to the frequency of cholelithiasis, and since these statistics have been collected in different parts of the world certain information can be obtained as to the racial and geographic distribution of the biliary calculi. In comparing the various statistics one meets with difficulties, however, because the material upon which they are based differs. Thus, some of the statistics have been collected from surgical clinics and laboratories while others make use of necropsies. This latter material, too, is not uniform. It is well known that below the age of twenty years, gallstones are very rare, and pathologic institutes with a large number of autopsies on children will, therefore, show a much lower percentage than institutions which draw their material from infirmaries restricted to aged and chronically disabled patients. A disadvantage of many statistics is the failure to differentiate between the principal types of gallstones, the pathogenesis of which is different. Little attention is usually given to the changes in the gallbladder which are of much significance.

Considering first the statistics from Central Europe, in particular those from Germany, Austria, and Switzerland, the percentage varies between 8.52 (Freiburg i. Br.) and 16.6 (Vienna) (Lotzin, W. Fischer, Kaufmann, Naunyn, v. Recklinghausen, Schretzenmayr, a.o.). The average is close to 10 per cent. Scheel reported from Denmark 11 per cent while the figures for Russia are much lower, namely 2.2 per cent (E. and M. Hesse). In Bosnia (Balkan) Kosti counted only 1.9 per cent. The British statistics speak of from 5.4 (Midlesex Hospital—Colwell) to 8.4 (Leeds—Gross) per cent.

*From the Department of Pathology of the Cook County Hospital.
Received for publication, October 12, 1932.

For North America I quote the following figures:

Mosher (Johns Hopkins)	6.94 per cent
Mitchell (Chicago-Coroner)	3.1 per cent
Ophuels (Stanford)	7.1 per cent
Ludlow (Cleveland)	6.97 per cent
Mentzer (Mayo)	20.09 per cent
Herter (New York Presbyterian)	7.6 per cent
Ryerson (Canada)	4.0 per cent

In Indian natives Rogers observed gallstones in 5.4 per cent. Ludlow, Mole and Van Gouder point to the rarity of gallstones among the Chinese (2.25 per cent) which also holds true for the Korean population (2.91 per cent). Statistics from Japan quote figures between 3.5 and 6.8 per cent (Miyake, Murakami, Kido).

The reports on the frequency of gallstones in the Negro race vary greatly. Garnier and Prieur assume that there are no differences in this respect between the white and the colored race. Bloch (New Orleans), Gould and Warren (Louisville), and Alden (Atlanta) are struck by the rare occurrence of biliary calculi in the Negro population of the Southern states. Mitchell did not find a case of cholelithiasis among 85 coroner autopsies on negro males. In the Canal Zone, Clark counted 2.2 per cent gallstones among negroes. In Cleveland, Ludlow found 3.08 per cent gallstones in the negroes and Mosher in Baltimore, 5.5 per cent. None of these statistics distinguishes between the different forms of stones.

In order to obtain additional information about the frequency of gallstones in the North American negro as compared to that in the white population, I have collected the data of 2621 autopsies on people over twenty years of age which were performed under my supervision between Jan. 1, 1929, and June 30, 1932. Special attention was given to the type of stone, the associated pathologic changes of the gallbladder and to the complications. The age groups below twenty were excluded since there was no case of cholelithiasis among them. The calculation of the percentage on the basis of the total number of autopsies (3633) would, therefore, have led to erroneous results.

Among the 2621 autopsies there were 208 cases with gallstones or 9.03 per cent. Arranged according to race, sex, and age the frequency of gallstones was as shown in Table I.

TABLE I

AGE	WHITE		COLORED	
	MALES	FEMALES	MALES	FEMALES
	Per Cent	Per Cent	Per Cent	Per Cent
21-30	0	5	0	3.28
31-40	1.45	11.51	0.73	7.50
41-50	4.24	21.90	0	12.24
51-60	6.75	17.07	1.86	16.27
61-70	11.20	29.03	4.34	25.0
Above 70	13.90	24.44	6.66	20.0
General Average	6.76	17.57	1.04	10.23

The most striking difference is found when one compares the figures for the white and colored males. They show that biliary calculi are six times as

common in the white males as in the negro males. For the female sex the difference is much less marked, the relation between the white and colored women amounting to 1.7:1. Practically all the statistics on gallstones stress the higher incidence in women pointing out that the stones are from 1.6 to 5 times more common in females than in males. It is only in Japan that such sexual differences do not seem to exist (Miyake and Ishiyama). In surgical statistics the difference between males and females is even greater than in the necropsy material, the relation amounting to 1:8. Garnier and Prieur suggest that in the females gallstones are more apt to cause trouble than in the males. In none of the statistics have I found such a great difference as in my group of colored patients. While the relation between the white males and females was 1:2.5 it was 1:10 for the colored patients.

As far as the age groups are concerned it appears that in both races gallstones occur earlier in the females than in the males in both sexes gradually increasing in frequency with progressing age.

In both races the mixed cholesterol-bile pigment-calcium stones were by far predominating, but the bile pigment stones were more common in the negroes than in the white people:

TYPE OF STONE	WHITE	COLORED
Mixed	85.18%	78.26%
Bile pigment	11.72%	17.38%
Cholesterol	3.08%	4.34%

According to Miyake and Ishiyama bile pigment stones are very common in Japan forming 65.19 per cent of the biliary calculi. In Dutch India, too, bile pigment stones are predominating (Langer).

There were no racial or sexual differences as to the number and size of the gallstones. Multiple stones were five times as common as single stones, the majority of the gallbladders containing from 10 to 25 stones. Two-thirds of the stones were from 3 to 15 mm. in diameter and only in eleven cases were the stones larger than 30 mm.

Eighty-nine (or 43 per cent) of the gallbladders with stones did not show any macroscopic changes save for an occasional decreased transparency of the wall as result of thickening of the subserous fibrous layer. Microscopically these gallbladders could not be called normal except those with pure cholesterol stones. There was a more or less marked thickening of the fibrous layer between serosa and muscularis which, in some instances, was combined with small perivascular round cell infiltrations. The muscle fibers were often slightly hypertrophic and were separated by an increased amount of connective tissue. The epithelial lined crypts of the mucosa often extended deeper into the wall than normally and the mucosa was atrophic.

The remaining 119 gallbladders revealed different forms of grossly visible changes which were classified into catarrhal cholecystitis, cholesterosis, ulcerative cholecystitis, suppurative cholecystitis, scarring of the mucosa, diffuse scarring of the wall and hydrops of the gallbladder. There were seven cases of carcinoma of the gallbladder. Among the two races and sexes the lesions of the gallbladder were distributed as shown in Table II.

TABLE II

	MACROSCOPICALLY UNCHANGED	CATARRHAL CHOLECYSTITIS	CHOLESTEROSIS	ULCERATIVE CHOLECYSTITIS	SUPPURATIVE CHOLECYSTITIS	SCARRING OF MUCOSA	SCARRING OF WALL	HYDROPS	CARCINOMA	
White Males	49	4	3	2	8	9	3	3	3	3 cases with surgical removal of the gallbladder
White Females	27	5	3	2	7	13	12	7	2	
Colored Males	4	1	–	–	–	–	1	–	–	
Colored Females	12	3	4	–	1	4	11	3	2	

Table II indicates that the gallbladders with slight and only microscopically visible lesions are more common in the male sex than in the female sex, while in the female sex the more severe chronic lesions predominate.

In 12.9 per cent of the cases of cholelithiasis stones were also present in the extrahepatic bile ducts, namely in the common duct 17 times, in the cystic duct 13 times, in the papilla 6 times and in the hepatic duct twice. There were two cases in which the wall of the gallbladder contained numerous small faceted stones which were impacted into diverticula-like outpouchings of the wall and protruded over the serosal surface.

Perforation of the gallbladder occurred 10 times. In four instances the gallbladder communicated with the first portion of the duodenum. In two female patients with cholecystoduodenal fistulas a large stone had entered the intestine causing death from intestinal obstruction. There were two cases of pericholecystic abscesses following perforation of the gallbladder. In one instance the gallbladder had broken into the liver and had caused the formation of a large liver abscess. In another case was found an abnormal communication of the pus and stone filled gallbladder with the common duct. These two types of perforation are considered as very rare by Rolleston and McNee. One gallbladder with a ruptured aneurysm of the cystic artery had perforated into the abdominal cavity with fatal hemorrhage into the latter (S. R. Rosenthal). There was also a case of biliary peritonitis in which the bile had escaped from the tremendously distended gallbladder through several, only microscopically visible openings.

It has often been stated that gallstones are the most important predisposing factor for malignant tumors of the gallbladder and extrahepatic bile ducts. In from 60 to 95 per cent of the cases of carcinoma of the gallbladder biliary calculi have been found. Gross found gallstones in 55.5 per cent of his cases of carcinoma of the extrahepatic bile ducts. The question, however, whether the stones are not secondary to the tumor formations still remains open. In my material 70 per cent of the cases of carcinoma of the gallbladder were associated with gallstones and only 16 per cent of the carcinomas of the extrahepatic bile ducts showed this combination. The total incidence of malignant tumors of the extrahepatic bile tract (gallbladder and ducts) among the patients with gallstones was 4.29 per cent which was four times the incidence of the patients without stones. Other statistics give figures between 1.04 and 12 per cent.

In 26 instances or 12.5 per cent of the cases with biliary calculi death was directly or indirectly due to the stones. The mortality rate was decidedly higher in the white patients than in the negroes the relative figures being 18.2 and 6.2 per cent. The most common cause of death was suppurative or ulcerative inflammation of the gallbladder (16 cases). Impaction of a stone in the papilla Vateri was next in frequency (6 cases). Two cases of intestinal obstruction by gallstones and a case each of fatal hemorrhage and biliary peritonitis have been previously mentioned. Because of the still unsettled question as to the relation between cholelithiasis and carcinoma of the gallbladder and extrahepatic bile ducts I have excluded these tumors in calculating the death rate for the biliary calculi.

Because of the frequency of gallstones as a casual finding, it will be interesting to investigate whether certain extrahepatic diseases are more often associated with stone formation in the gallbladder than others. There are, especially, three conditions which have often been linked with biliary calculi, since they seem to bear evidences of a disturbed cholesterol metabolism, namely atheromatosis, carcinoma, and diabetes mellitus. Mosher found atheromatosis in 45 per cent of his cases of cholelithiasis and Chauffard observed this combination in 26.5 per cent. In Gross' material the general percentage of atheromatosis was 51.8 per cent while it was 71.8 per cent in the cases of gallstones. Pure cholesterol and mulberry stones were much more often combined with atheromatosis than the mixed stones. I am giving a list of the frequency of atheromatosis in the patients with and without gallstones, which shows that it is only in the males that slight differences can be noted (Table III).

TABLE III

FREQUENCY OF ATHEROMATOSIS IN PATIENTS

	WITH GALLSTONES	WITHOUT GALLSTONES
White males	44.9%	32.0%
White females	20.9%	21.7%
Colored males	33.3%	19.2%
Colored females	17.5%	18.6%

Musser stated that malignancy anywhere in the body favors the formation of stones in the gallbladder and also Schretzenmayr and Lotzin point to the frequent combination of gallstones with carcinomas outside the biliary tract. Gross, on the other hand, says if anything patients with biliary calculi are slightly less liable to cancer than normal persons. Among my cases of cholelithiasis there were 40 incidences of carcinoma outside the biliary tract or 20 per cent as compared to 18 per cent in the other cases. This difference is much too insignificant as to indicate a greater disposition of the patients with gallstones to carcinoma. In this connection it may be mentioned that there exists a certain antagonism between atheromatosis and carcinoma (Casper). There were four cases of diabetes mellitus with gallstones (8.3 per cent) which is in accordance with the statement made by Rolleston and McNee that diabetes has no tendency to produce cholelithiasis (see, however, Aschoff and Gross).

Obesity has often been considered as an important predisposing factor for biliary calculi. Gross found the average body weight of the patients with gallstones to be decidedly higher than that of the patients free from stones. I refrain from giving figures of the body weight because autopsy material is of no significance in this regard.

Of the other pathologic changes, those of the liver and pancreas are of importance. Biliary cirrhosis was observed in 6 cases or 3 per cent, and atrophic cirrhosis was found three times. Dufour has recently pointed to the rare combination of alcoholic cirrhosis with cholelithiasis for which he advances the odd theory that the alcohol may keep the cholesterol in solution. There were only three cases of pancreas apoplexy among the 208 patients with gallstones. The frequency of cholelithiasis in patients with cardiac diseases stressed by Brockbank is not evident in my material. I cannot comment on the statement made especially in the British literature that gallstones are particularly common among inmates of psychopathic hospitals (Candler, Flemming, Keay, a.o.) since my observations are too limited in this regard.

DISCUSSION

The statistics presented in this paper indicate that in the white population of Chicago cholelithiasis is more common than in the negroes. This racial difference manifests itself chiefly in the males while in the females it is much less striking. Three factors have been associated with the etiology of the most common and most important type of gallstones, the mixed cholesterol-bile pigment-calcium stone, namely infection, biliary stasis and diets rich in fat. It has been said that the low incidence of biliary calculi in certain parts of the world is due to the vegetarian diet and strenuous outdoor life of their population. The frequency of gallstones in patients with heart diseases or depressive psychosis has been attributed to their sedentary life which favors biliary stasis.

The material upon which my observations are based is uniform and I do not think that there are sufficient differences in the diet, occupation, or mode of living between the white and colored patients of the large charity hospital of Cook County as to account for the greater disposition to biliary calculi in the white patients. It is difficult to conceive that infections of the gallbladder should be more common in the white race than in the colored race, no other infection showing such racial differences. The inability to find a satisfactory explanation in the light of the most favored theories on the formation of gallstones draws attention to additional factors which have been recently brought out by Westphal and Aschoff, namely the importance of nervous and endocrine influences upon the tonus and the emptying of the gallbladder. In the female sex the predisposing functional influences are apparently so marked that they compensate for the racial differences. Observations on the living patients, especially comparative studies on the emptying of the gallbladder between white individuals and negroes will have to decide whether the suggestions made on the basis of anatomical findings can be maintained.

SUMMARY

A statistical study on the frequency of gallstones in the white and colored patients of a large charity hospital shows that in the white males these stones are much more common than in the negroes. In females this difference is much less pronounced. The various theories on the etiology of the gallstones are briefly discussed in the light of these findings.

REFERENCES

Alden, H. S.: Cholelithiasis and Cholecystitis in Negro, South. M. J. **20:** 828, 1927.
Aschoff, L.: Die Gallensteine, Beihefte z. Med. Klin. No. 3, 1931.
Bloch, E.: Cholelithiasis in the Negro, Surg. Gynec. Obst. **43:** 465, 1926.
Brockbank: Quoted by Rolleston and McNee.
Candler: Quoted by Rolleston and McNee.
Casper, J.: Boesartige Geschwuelste und Artesiosklerose, Ztschr. f. Krebsforsch. **36:** 354, 1932.
Clark: Quoted by Bloch.
Colwell: Quoted by Rolleston and McNee.
Dufour, H.: Grand éthylism du foie. Lithiase vésiculaire, Bull. et mém. Soc. méd. d hôp. de Paris **55:** 377, 1931.
Fischer, W.: Einige Beobachtungen an entzuendeten Steingallenblasen, Beitr. z. path. Anat. u. z. allg. Path. **84:** 535, 1930.
Fleming, G. W.: Cholelithiasis in the Insane, J. Path. & Bact. **33:** 197, 1930.
Gould and Warren: Quoted by Bloch.
Garnier, M., and Prieur, R.: Affections des voies biliaires, in Roger, G. H., Widal, F., et Teissier, P. J.: Nouveau Traite de Medecine, Fasc. XVI. Pathologie du Foie et des Voies Biliaires, Masson, Paris, 1928.
Gross, D. M. D.: A Statistical Study of Cholelithiasis, J. Path. & Bact. **32:** 503, 1929.
Herter: Quoted by Mitchell.
Hesse, E., and Hesse, M.: Ueber die Haeufigkeit der Gallensteine auf Grund eines Sektionsmateriales von 17 402 Faellen, Beitr. z. klin. Chir. **89:** 611, 1914.
Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, ed. 7 and 8, 1922.
Keay: Quoted by Fleming.
Kosti: Quoted by Miyaki and Ishiyama.
Lotzin, R.: Uber die Beziehungen der Gallensteine zum Krebs der extrahepatischen Gallenwege, zugleich ein Beitrag zur Lehre von der Gallensteinwanderung und dem Hydrops der Gallenwege, Arch. f. klin. Chir. **139:** 525, 1926.
Ludlow, A. I.: Cholelithiasis in the Korean, Surg. Gynec. Obst. **50:** 51, 1930.
Mentzer, S. H.: A Clinical and Pathological Study of Cholecystitis and Cholelithiasis, Surg. Gynec. Obst. **42:** 782, 1926.
Mitchell, L. G.: The Incidence of Calculi in the Gall-bladder as Met With in 1600 Autopsies, Ann. Surg. **68:** 289, 1918.
Miyake, H.: Statistische, klinische und chemische Studien zur Aetiologie der Gallensteine, Arch. klin. Chir. **101:** 54, 1925.
Miyake, H., and Ishyama, F.: Statistische und klinische Studien auf Grund von 754 Faellen von Cholelithiasis bei Japanern, Deutsche Ztschr. f. Chir. **225:** 187, 1930.
Murakami: Quoted by Aschoff.
Musser, J. H.: Primary Carcinoma of the Gall Bladder and the Bile Ducts, Boston M. & S. J. **121:** 525, 1889.
Ophuels, W.: A Statistical Survey of Three Thousand Autopsies from the Department of Pathology of Stanford University Medical School, Stanford Univ. Press **1:** 25, 1926.
Rogers, L.: Pathological Evidence Bearing on Disease Incidence in Calcutta, Glasgow M. J. **103:** 1, 1925.
Rolleston, H., and McNee, J. W.: Diseases of the Liver, Gall-Bladder and Bile Ducts, ed. 3, London, 1929, MacMillan and Co.
Rosenthal, S. R.: Ruptured Aneurysm of the Cystic Artery of the Gallbladder as a Result of Toxic Arteritis, Arch. Path. **11:** 884, 1931.
Ryerson, E. S.: The Aetilogy of Cholelithiasis, With Special Reference to Age Incidence, Canad. M. A. J. **1:** 832, 1911.
Schretzenmayr, A.: Gallensteinleiden und Konstitution, Ztschr. f. Konstitutions Lehre **13:** 788, 1928.

A STUDY OF THE TOTAL AND ULTRAFILTRABLE CALCIUM AND THE ACID-SOLUBLE PHOSPHATE CONTENT OF THE BLOOD SERUM OF FOUR HUNDRED AND TWENTY-TWO HEALTHY CHILDREN*

MARION SMOOT NEEDELS, A.B., AND CARL MAURITZ MARBERG, PH.D.
CHICAGO, ILL.

A STUDY of the calcium and acid-soluble phosphate content of blood serum of a large group of children might be of some value because of the possible relationship between these values, calcium and phosphorus metabolism, and bone development. It was also thought that there might be a relationship between the incidence of dental caries and the calcium and acid-soluble phosphate content of the blood serum. The work to be reported in this paper is part of an extensive nutritional study undertaken on 422 children at Mooseheart, Illinois by the Otho S. A. Sprague Memorial Institute, the University of Chicago, and the Chicago Dental Research Club under the direction of Milton Theo. Hanke.[1]

There were two groups of children; namely, a test group (323) and a control group (99). The test group was ingesting the standard Mooseheart diet for one year (control period) and received a pint of orange juice and the juice of one lemon per day in addition to the standard Mooseheart diet during the second year (test period). The control group received only the standard diet. The children were all living under identical conditions and they were all in good health. The total calcium and acid-soluble phosphate content of the blood serum were determined at six-month intervals during a two-year period on the test group (5 determinations per child) and during a one-year period on the control group (2 determinations per child). The ultrafiltrable calcium content of the blood serum was determined for all of the children (422) during a one-year period (2 determinations per child).

EXPERIMENTAL METHODS

Treatment of the Sample.—Blood (20 c.c.) was drawn from the median basilic vein of each of the children at six-month intervals. The blood was immediately defibrinated in a small pyrex beaker by gently stirring with a glass rod. The defibrinated blood was promptly placed in 15 c.c. pyrex centrifuge tubes and centrifuged for fifteen minutes, at about 1500 revolutions per minute. The clear serum was immediately removed by means of a pipette, transferred to a small pyrex tube, and transported to the laboratory.

Determination of Total Calcium and Acid-soluble Phosphate.—Exactly 3 c.c. of the serum was slowly pipetted into 12 c.c. of 10 per cent trichloracetic

*From the Otho S. A. Sprague Memorial Institute and the Department of Pathology, University of Chicago, Chicago, Illinois.
Received for publication, Oct. 6, 1932.

acid solution with constant agitation. The mixture was allowed to stand for fifteen or twenty minutes, and then filtered through a Whatman No. 40 filter paper. The clear filtrate was used for the determination of total calcium and acid-soluble phosphate.

Calcium was determined by the method of Roe and Kahn[2] with the following modifications: Centrifugalizations were conducted for four minutes instead of two minutes as recommended by Roe and Kahn. This produces a firm mat of tricalcium phosphate which is not disturbed by the subsequent decantation and drainage. We have found it advisable to allow twenty to twenty-five minutes for drainage instead of two minutes as recommended by Roe and Kahn.

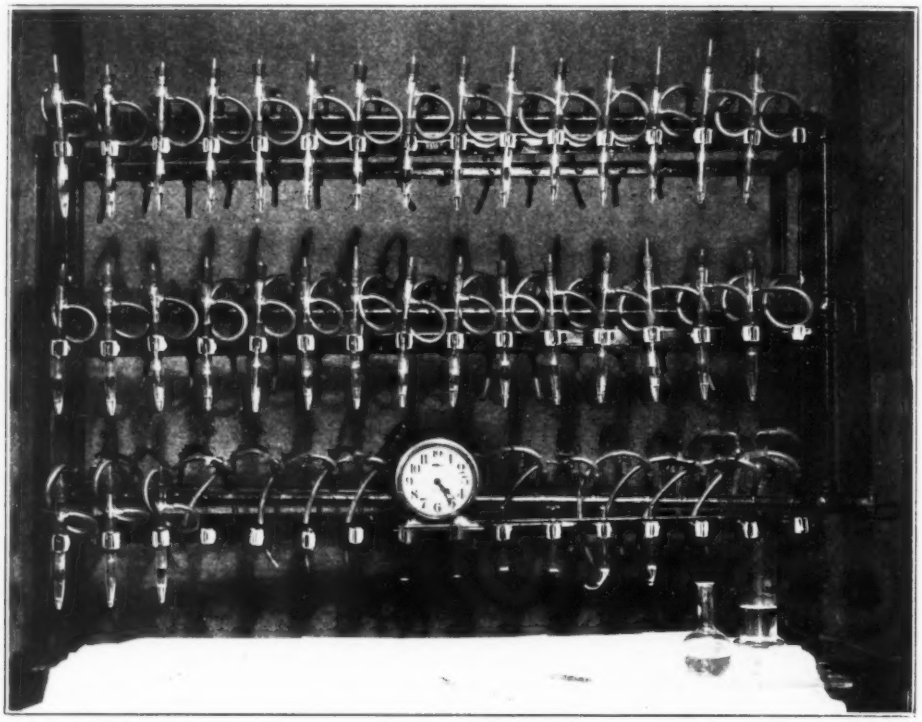


Fig. 1.—View of the ultrafiltration rack, showing also, in the lower rack, the method of formation of the membranes.

The technic and the quality of the reagents were checked each week by conducting an assay on a standard solution of $CaCl_2$. Powdered calcite, 62.500 mg., that has been dried for three hours at 110° C., is dissolved in the theoretical amount of dilute HCl. The solution is diluted to 250 c.c. in a Normax volumetric flask and preserved with toluene. This standard calcium solution contains 0.1 mg. calcium per c.c. For the test, measure 1.00 c.c. of this solution (by pipette) into a 15 c.c. graduated Pyrex centrifuge tube. Add 4 c.c. of a 10 per cent solution of trichloracetic acid. Proceed from this point as in determining the calcium content of serum. The greatest average inaccuracy of this method is 3 per cent.

Acid-soluble phosphate was determined by the method of Fiske and Subbarow[3] on a 5 c.c. portion of the trichloracetic acid filtrate. The color was

developed with 1, 2, 4—aminonaphtholsulphonic acid; but this was prepared according to Roe and Kahn. There were no other modifications. The error appears to be largely colorimetric and seldom exceeds 2 per cent.

Determination of Ultrafiltrable Ca.—The method used for the determination of the ultrafiltrable calcium content of the sera is a modification of the methods of Moritz[4] and of Greenberg and Gunther.[5]

Apparatus.—The units of apparatus were exactly the same as those employed by Moritz. These were arranged in a specially constructed rack capable of holding 90 such units (Fig. 1). These units were then connected to the vacuum lines by means of rubber tubing equipped with pinch clamps. The vacuum lines were connected to a 30-gallon reservoir. A negative pressure of 150 mm. Hg. is maintained in the 30-gallon reservoir by means of a Cenco

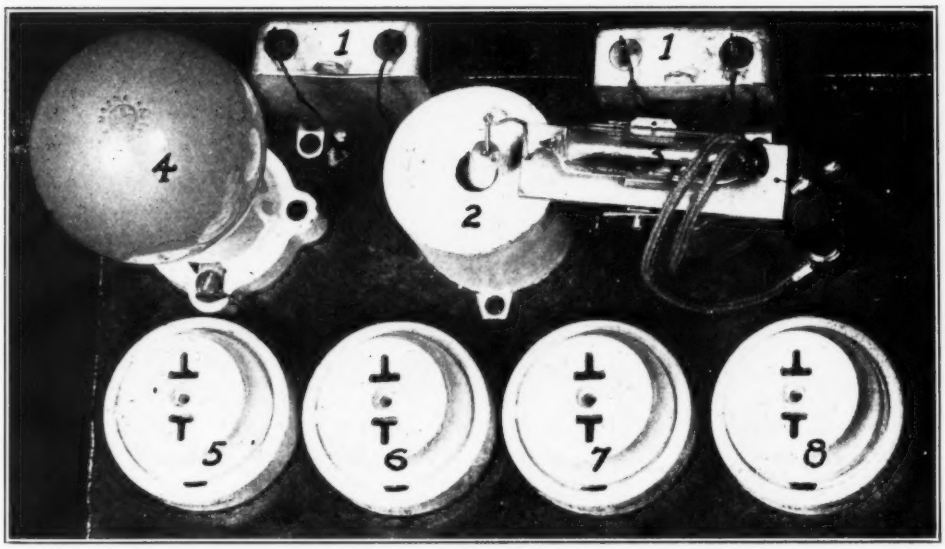


Fig. 2.—"Relay" *1*, 1 mfd. condenser, one in each circuit; *2*, solenoid—¼ lb. B & S. No. 28 copper wire; *3*, "Time-O-Stat" mercury contactor; *4*, 40 W. bulb, in series with manometer contacts and solenoid; *5*, connection to manometer contacts; *6*, connection to 110 v. D. C. manometer circuit; *7*, connection to 110 v. A. C., pump circuit; *8*, connection to Cenco "Megavac" pump.

Megavac pump. Operation of the pump is controlled by a specially designed relay that is illustrated in Fig. 2. The pressure variation was never more than ±0.5 mm. mercury.

Membranes.—We found it difficult, at first, to prepare membranes that were uniformly permeable. The method finally adopted, which appears to be quite satisfactory and which has enabled us to prepare membranes of uniform permeability repeatedly, was as follows: Soluble cotton (nitrocellulose) which was purchased from Charles Cooper & Co., New York City, was plucked by hand and allowed to air-dry overnight. It was dried at about 40° C. for twelve or more hours. One hundred grams of this material was placed in a 2-liter flask, 500 c.c. of 95 per cent alcohol was added (the alcohol is prepared by dilution of absolute alcohol with distilled water), and then 500 c.c. of absolute ethyl

ether. This mixture was agitated until all of the soluble cotton had dissolved. The mixture was allowed to stand until the insoluble suspended particles had settled and then the clear supernatant liquid was decanted into 200 c.c. Erlenmeyer flasks, which were kept tightly stoppered until used.

The membranes are formed by pouring the soluble cotton solution in a small stream into 10 by 100 mm. pyrex test tubes. Two test tubes are filled at a time. The tubes are inverted and rotated until they have almost ceased to drip; they are clamped into position as illustrated in the lower rack of Fig. 2; the time is recorded and the partially solidified drippings are removed from the outlet of the test tube, from time to time, with a pair of forceps. It is imperative that the outlet be kept open throughout the drying period of exactly ten minutes. Remove the tubes from the rack and immerse in triple distilled water for thirty minutes. The transparent membranes are removed from the tubes, tested for leaks (by filling with triple distilled water, closing the orifice, and applying pressure), and cut to a length of 70 mm. The finished membranes are filled with and immersed in triple distilled water and placed in the refrigerator overnight. They are ready to use on the following day.

The Ultrafiltration Process.—A membrane is removed from the dish and pressed in folds of clean white surgical gauze. The collapsed tube is reopened by directing a sharp forceful expiration at the orifice of the tube. Introduce exactly 3 c.c. of blood serum into the membrane. Lubricate stopper R* of the ultrafiltration unit by applying a small amount of a 5 per cent solution of soluble cotton in alcohol-ether. Slip the membrane into place and tie with white silk thread. Attach the empty 15 c.c. graduated pyrex centrifuge tube and clamp the apparatus into place on the rack.

It was necessary, in this investigation, to conduct a large number of ultrafiltrations. The ultrafiltration units were, therefore, collected on the rack under atmospheric pressure and evacuated as a group. Ultrafiltration is continued for four hours. The filtrates should be clear and colorless and usually have a volume of 1.7 to 1.8 c.c. Turbidity or color indicates leakage.

Determination of Calcium and Acid-soluble Phosphate in the Ultrafiltrate.—Add 12 c.c. of a 10 per cent solution of trichloracetic acid to the ultrafiltrate and dilute to 15 c.c. with triple distilled water. Mix thoroughly by repeated inversion against a clean rubber thumbcot. The calcium and phosphate content of this liquid is determined on 5 c.c. portions, using the methods previously described.

Calculation of the Ultrafiltrable Calcium Value.—Rona and Takahashi[6] have shown that all of the phosphate of horse serum is diffusible by compensation dialysis. Neuhausen and Pineus[7] have shown that all of the phosphate of hog serum is ultrafiltrable. Pineus, Peterson, and Kramer[8] state that human serum phosphate is completely diffusible. We have conducted experiments which show that the acid-soluble phosphate of human serum is completely ultrafiltrable. We make use of this fact in the calculation of the ultrafiltrable calcium content of the serum.

*See diagram of Moritz (loc. cit.)

We have determined the total calcium and acid-soluble phosphate content of the serum and the calcium and phosphate content of the ultrafiltrate. All of the acid-soluble phosphate and a certain amount of the calcium are ultrafiltrable. The ultrafiltrate will, therefore, contain a given fraction of the total acid-soluble phosphate and should contain an identical fraction of the total ultrafiltrable calcium. The total ultrafiltrable calcium can, therefore, be readily calculated from the formula:

$$\frac{\text{Total P}}{\text{P in ultrafiltrate}} \times \text{Ca in ultrafiltrate} = \text{Total ultrafiltrable Ca.}$$

This method of calculation avoids empiric corrections for the amount of water introduced by the moist membrane, and for the volume of the proteins and other nonultrafiltrable substances that are present in the serum, because any effect that these substances may have would be equally applicable to the phosphate and to the calcium.

RESULTS

Determinations of the total calcium and acid-soluble phosphate of the blood serum of private dental patients indicated that the phosphate content of the serum became lower as a child reached maturity and the calcium content of the serum appeared to show some seasonal variation. Table I is a summary of our Mooseheart data arranged so as to show variations of the calcium and phosphate with age, and in the spring and fall of the respective years.

The calcium values, while the children were ingesting the standard Mooseheart diet, were rather consistently lower in spring than they were in fall. A

TABLE I

TOTAL CALCIUM AND ACID-SOLUBLE PHOSPHATE (CALCULATED AS P) CONTENT OF THE BLOOD SERUM OF 322 CHILDREN CHARTED BY AGE GROUPS

AGE OCTOBER, 1929	NUMBER OF CHILDREN	STANDARD MOOSEHEART DIET						STANDARD MOOSEHEART DIET PLUS 1 PINT ORANGE JUICE AND JUICE OF 1 LEMON PER DAY			
		OCTOBER, 1929 TOTAL		APRIL, 1930 TOTAL		OCTOBER, 1930 TOTAL		APRIL, 1931 TOTAL		OCTOBER, 1931 TOTAL	
		Ca MG. %	P MG. %	Ca MG. %	P MG. %	Ca MG. %	P MG. %	Ca MG. %	P MG. %	Ca MG. %	P MG. %
Boys											
10	7	10.79	4.60	10.40	4.58	10.87	4.27	10.36	4.70	10.53	4.59
11	14	10.48	4.67	10.09	4.61	10.74	4.50	10.85	4.68	10.89	4.49
12	17	10.46	4.50	10.07	4.55	11.05	4.51	10.75	4.87	10.93	4.68
13	23	10.41	4.65	10.14	4.76	10.77	4.44	11.05	4.76	10.62	4.50
14	16	10.91	4.88	10.22	4.75	10.88	4.22	10.89	4.60	10.68	4.28
15	16	10.02	4.81	9.98	4.86	10.76	4.38	10.69	4.19	10.49	3.92
16	17	10.52	4.77	10.10	4.59	10.79	4.26	10.82	4.18	10.89	3.83
Girls											
11	4	10.72	4.80	10.52	4.80	10.43	4.44	10.48	4.56	10.74	4.25
12	28	11.03	4.54	10.39	4.57	10.76	4.62	10.39	4.39	10.68	4.11
13	39	10.83	4.46	10.43	4.43	10.98	4.28	10.36	4.11	10.65	3.93
14	46	11.13	4.23	10.32	4.17	10.99	4.16	10.46	3.99	10.68	3.92
15	59	11.16	4.16	10.24	4.07	11.00	4.10	10.46	3.89	10.66	3.78
16	27	10.80	3.89	10.13	3.99	10.66	3.88	10.65	3.82	10.47	3.78
17	9	10.44	4.01	10.17	3.95	10.99	3.89	10.23	3.84	10.30	3.98

seasonal variation in the calcium content of the serum did not occur in the case of the boys and was not very definite in the case of the girls, when orange and lemon juice was added to the daily diet. The acid-soluble phosphate content of the blood serum appears not to be subject to seasonal variations. The phosphate value is definitely lower in the more mature age groups. The values in the table suggest a fairly smooth decline in the phosphate value with increasing age in the case of the girls and no change at all in the case of the boys until they have reached the age of seventeen. Even then the decrease is not very definite in the case of the boys.

The results obtained particularly by Mellanby and Pattison[9] indicate that a correlation may exist between calcium and phosphorus metabolism and dental caries. We have attempted to correlate the total calcium and acid-soluble phosphate values of the blood serum with the incidence of dental caries. The results are tabulated in Table II.

TABLE II

AN ATTEMPT TO CORRELATE THE TOTAL CALCIUM AND ACID-SOLUBLE PHOSPHATE (CALCULATED AS (P) CONTENT OF THE BLOOD SERUM WITH THE INCIDENCE OF DENTAL CARIES

	NUMBER OF CHILDREN	TOTAL Ca MG. %	TOTAL P MG. %
End of Control Period (October, 1930) (Standard Moosecheart diet)			
Never any caries	15	10.52	4.53
No caries for one year	52	10.94	4.28
Active progressive caries	256	10.90	4.22
End of Test Period (October, 1931) (Standard Moosecheart diet plus 16 oz. orange juice and the juice of 1 lemon per day)			
Never any caries	15	10.60	4.30
No caries for two years	35	10.62	4.10
Arrested caries	161	10.65	4.04
Active progressive caries	112	10.71	3.99

The values for the group marked "Never any caries" can hardly be taken as representative because there were only 15 children in this group and the individual differences were great. The values for the other groups are practically identical for any given period. We must conclude, therefore, that there is no correlation between the total calcium and acid-soluble phosphate content of the blood serum and dental caries.

It was, of course, possible that the ultrafiltrable calcium had undergone changes even though the total calcium value was identical regardless of the incidence of dental caries. We decided, therefore, to determine the amount of ultafiltrable calcium and these determinations were conducted on all of the children during the second year of the study period. Three hundred and twenty-three of these children were receiving orange and lemon juice in addition to the standard diet during this period, 99 of the children were ingesting the standard diet but did not receive any additional citrus fruit juice. The values are summarized in Table III. It is quite obvious, from this table, that there was no

TABLE III

AN ATTEMPT TO CORRELATE THE TOTAL AND ULTRAFILTRABLE CALCIUM CONTENT OF THE BLOOD SERUM WITH THE INCIDENCE OF DENTAL CARIES

	NUMBER OF CHILDREN	APRIL, 1931 Ca IN MG. % TOTAL	APRIL, 1931 Ca IN MG. % ULT.	OCTOBER, 1931 Ca IN MG. % TOTAL	OCTOBER, 1931 Ca IN MG. % ULT.
Test Group (Standard Mooseheart diet plus 16 oz. orange juice and the juice of 1 lemon per day)					
Never any caries	15	10.57	5.70	10.60	6.08
No caries for two years	35	10.63	5.95	10.62	6.03
Arrested caries	161	10.60	5.81	10.65	5.99
Active progressive caries	112	10.52	5.85	10.71	6.07
Control Group (Standard Mooseheart diet. No additional orange or lemon juice)					
Never any caries	3	10.50	5.53	10.41	6.76
No caries for six months	29	10.41	5.62	11.20	6.11
Active progressive caries	67	10.29	5.78	11.14	6.10

correlation between the total or ultrafiltrable calcium content of the serum and the incidence of dental caries.

A comparison of the test group with the control group shows that the ingestion of orange and lemon juice had no effect upon the ultrafiltrable calcium content of the blood serum.

SUMMARY

1. The total and ultrafiltrable calcium and the total acid-soluble phosphate content of the blood serum of a large group of children is reported.

2. There were two groups of children; namely, a test group (323) and a control group (99). The test group were ingesting the standard Mooseheart diet for one year (control period) and received a pint of orange juice and the juice of one lemon per day in addition to the standard Mooseheart diet during the second year (test period). The control group received only the standard diet. The children were all living under identical conditions and they were all in good health. The total calcium and acid-soluble phosphate content of the blood serum was determined at six-month intervals during a two-year period on the test group (5 determinations per child) and during a one-year period on the control group (2 determinations per child). The ultrafiltrable calcium content of the blood serum was determined for all of the children (422) during a one-year period (2 determinations per child).

3. The calcium values, while the children were ingesting the standard diet, were rather consistently lower in spring than they were in fall. A seasonal variation in the calcium content of the serum did not occur in the case of the boys and was not very definite in the case of the girls when a pint of orange juice and the juice of one lemon was added to the daily diet.

4. The acid-soluble phosphate content of the blood serum appears not to be subject to seasonal variations but is definitely lower in the more mature age groups.

5. There is no correlation between the total or ultrafiltrable calcium or total acid-soluble phosphate content of the blood serum and the incidence of dental caries.

REFERENCES

1. Detailed reports of the dental and nutritional aspects of this study are to appear elsewhere.
2. Roe, J. H., and Kahn, B. S.: The Colorimetric Determination of Blood Calcium, J. Biol. Chem. **81**: 1, 1929.
3. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. **66**: 375-400, 1925.
4. Moritz, A. R.: The Effect of Ultraviolet Irradiation on the State of Serum Calcium, J. Biol. Chem. **64**: 81, 1925.
5. Greenberg, D. M., and Gunther, Lewis: On the Determination of Diffusible and Non-diffusible Serum Calcium, J. Biol. Chem. **85**: 491, 1930.
6. Rona, P., and Takahashi, D.: Beitrag zur Frage nach dem Verhalten des Calcium im Serum, Biochem. Ztschr. **49**: 370, 1913.
7. Neuhausen, B. S., and Pincus, J. B.: A Study of the Condition of Several Inorganic Constituents of Serum by Means of Ultrafiltration, J. Biol. Chem. **57**: 99, 1923.
8. Pincus, J. B., Peterson, H. A., and Kramer, B.: A Study by Means of Ultrafiltration of the Condition of Several Inorganic Constituents of Blood Serum in Disease, J. Biol. Chem. **68**: 607, 1926.
9. Mellanby, May, Pattison, C. L., and Proud, J. W.: The Effect of Diet on the Development and Extension of Caries in Teeth of Children, Brit. Med. J. **2**: 354-5 (Aug. 30), 1924.

   Mellanby, May: Some Factors of Diet Influencing Spread of Caries in Children, Brit. Dent. J. **47**: 1045-57 (Oct. 1), 1926.

   Pattison, C. L.: Dietetic Conditions Which Influence the Calcium Content of Saliva, Brit. Med. J. **2**: 6 (July 3), 1926.

   Mellanby, May, and Pattison, C. L.: Action of Vitamin D in Preventing Spread and Promoting Arrest of Caries in Children, Brit. Med. J. **2**: 1079-82 (Dec. 15), 1928.

   Mellanby, May: Diet and Teeth: An Experimental Study. Part I. Dental Structure in Dogs. Special Report Series No. 140. Medical Research Council, London, 1929. Diet and Teeth: An Experimental Study. Part II. *A*. Diet and Dental Disease. *B*. Diet and Dental Structure in Mammals Other Than the Dog. Special Report Series No. 153. Ibid., 1930. The Influence of Diet on Caries in Children's Teeth (Interim Report). Special Report Series No. 159. Ibid., 1931.

A STUDY OF THE DIMETHYLAMINOBENZALDEHYDE TEST FOR ATROPINE*†

ARLIE A. O'KELLY, PH.D., AND CHARLES F. POE, PH.D., BOULDER, COLO.

A NUMBER of investigations have shown that some of the color tests for the various alkaloids are not specific. Levine[1] has reported that many phenols give the same color reaction with selenious-sulphuric acid reagent as do some of the alkaloids. Levine and Magiera[2] found the same to be true for the formaldehyde-sulphuric acid reaction for the opium alkaloids. Poe and coworkers[3] have found a number of organic compounds which give characteristic color reactions with different alkaloidal reagents.

One of the most recent tests proposed for atropine is the use of para-dimethylaminobenzaldehyde dissolved in sulphuric acid. Atropine, when treated with this reagent and heated, gives a red color which soon changes to a reddish violet. The test was proposed by Wasicky[4] in 1915. He tested a number of alkaloids, and found that the same test was given by hyoscyamine and scopolamine. The colors obtained by several other alkaloids were recorded. Ekkert[5] confirmed the work of Wasicky.

No record could be found where various organic compounds other than a few alkaloids had been tested with this reagent. The object, therefore, of the investigation reported in this paper was to test a large number of organic compounds with the dimethylaminobenzaldehyde reagent in order to determine whether or not there was any great number of substances which would give the atropine test, and whether this test was characteristic of any given organic grouping or class of organic compounds. Also, these compounds were mixed in varying amounts with atropine, and the test applied in order to determine which substances would interfere with the reaction.

PROCEDURE

Preparation of para-Dimethylaminobenzaldehyde Reagent.—Two grams of the aldehyde were dissolved in 6 gm. of chemically pure concentrated sulphuric acid, then 0.4 c.c. of water was added.

Preparation of Standard Atropine Solution.—A solution was prepared in alcohol containing one milligram of atropine per cubic centimeter.

Preparation of Solutions to Be Tested.—The compounds to be tested were dissolved in the proper solvent so that 1 c.c. of the solution was equivalent to 1 milligram of the compound. Care was taken in selecting a suitable solvent which would not only readily dissolve the compound, but would also volatilize on the water-bath.

*From the Chemistry Departments, Tennessee State Teachers College and University of Colorado.

Received for publication, October 10, 1932.

†Presented before the Denver meeting, American Chemical Society, August, 1932; released for publication September 19, 1932.

Procedure of Tests.—Three tests were made on each compound. For the first test, 1 c.c. of the solution to be tested was evaporated to dryness and then treated with two or three drops of the reagent, warmed on the water-bath for five minutes, and any color change was recorded. Any colors which developed before heating were also recorded. In the tables these are indicated with the words, "in cold." In the second test, 1 c.c. of the solution to be tested and 1 c.c. of standard atropine solution were mixed and the test made as described above. If there was no interference, a reddish violet color resulted. The third test was carried out as above, except that 5 c.c. of the solution of the organic compound were used with 1 c.c. of the atropine solution.

The results of the tests when the organic compounds were treated with the reagent are given in the pages immediately following. The name of the organic compound is given first, and directly opposite is given the color reaction. In cases where no color other than that caused by the reagents was developed, the compounds are listed with the statement, "No color reaction."

ALKALOIDS AND ALKALOIDAL SALTS

Organic Substances	*Color Reaction*
Apomorphine	Dark brown
Atropine	Reddish violet
Belladonnine	Violet in cold
Berberine	Brownish green
Brucine	Red
Cryptopine	Green
Daturine	Brownish violet
Delphinine	Brown
Emetine	Dark brown
Heroine	Brownish violet
Hyoscyamine	Reddish violet
Morphine	Rose violet to brown
Narceine	Orange
Narcotine	Brown
Papaverine	Orange
Piperine	Greenish brown
Quinidine	Brown, trace violet
Sanguinarine	Orange brown
Scopolamine	Rose violet
Solanine	Reddish violet in cold
Veratrine	Brownish violet

No Color Reaction.—Aconitine, caffeine, cinchonidine, cinchonine, cocaine, codeine, colchicine, cotarnine hydrochloride, dionine, ergotine, beta-eucaine hydrochloride, gelseminine, homatropine, hydrastine, nicotine, pelletierine, phenacaine, physostigmine, pilocarpine, pseudopelletierine, quinine, sparteine, strychnine, theobromine, theophylline.

AMINO ACIDS AND DERIVATIVES

Acetylphenylglycine	Brown, trace violet

No Color Reaction.—Alpha-Alanine, para-aminophenylglycine, arginine, asparagine, aspartic acid, *dl*-benzoylalanine, betaine hydrochloride, creatine, creatinine, diiodotyrosine, edestine, ethylglycollate, glutamic acid, glycine, glycyltryptophane, hippuric acid, isoleucine, leucine, para-nitrophenylglycine, alpha-phenylalanine, beta-phenylalanine, phenylglycine, tryptophane, tyrosine, *dl*-valine.

ALIPHATIC ACIDS

Malic acid	Brown, trace of violet
Monochloroacetic acid	Green

No Color Reaction.—Adipic acid, alpha-bromopropionic acid, beta-bromopropionic acid, formic acid, fumaric acid, levulinic acid, maleic acid, malonic acid, mucic acid, palmitic acid, propionic acid, stearic acid, succinic acid, tartaric acid, trichloracetic acid.

ALIPHATIC ACID SALTS, ESTERS, AND DERIVATIVES

No Color Reaction.—Ethyl oxalate, ethyl succinate, isobutyl acetate, isobutyl isothiocyanate, methyl isothiocyanate, sodium formate, sodium oxalate, tributyrin.

ALIPHATIC ALCOHOLS AND KETONES

Isoamyl alcohol	Dirty violet
Isobutyl alcohol	Light green
Melissyl alcohol	Brownish violet

No Color Reaction.—Acetylacetone, amyl alcohol, cetyl alcohol, dulcitol, erythritol, ethylene glycol, isopropyl alcohol, mannitol, methyl heptenone, octyl alcohol, terbutyl alcohol.

SUGARS

Arabinose	Greenish black
Galactose	Black
Melezitose	Reddish brown
Xylose	Black

No Color Reaction.—Glucose, lactose, levulose, maltose, *d*-mannose, raffinose, rhamnose, sucrose.

UREA AND URIC ACID DERIVATIVES

Allantoine	Green in cold
Barbituric acid	Brown
Triphenylguanidine	Dirty violet to black

No Color Reaction.—Allylphenylthiocarbamide, amytal (isoamylethylbarbituric acid), acetylmethylurea, alloxantine, barbital (diethylmalonylurea), biuret, *dl*-*n*-butylthiourea, dibromobarbituric acid, diphenylthiourea, guanine hydrochloride, ipral (calcium ethylisopropylbarbiturate), luminol (phenylethylbarbituric acid), peralga (aminopyrinediethylbarbiturate), thiobarbituric acid, thiourea, urea, urethane, uric acid.

GLUCOSIDES

Aesculin	Dark brown
Colocynthin	Brown
Elaterin	Brown
Phloridzin	Orange
Picrotoxin	Brown, trace of violet
Salicin	Reddish violet in cold
Santonin	Brown

No Color Reaction.—Amygdalin, arbutin, convallamarin, digitalin, saponin.

MISCELLANEOUS ALIPHATIC COMPOUNDS

No Color Reaction.—Acetal, acetaldoxime, acetamide, acetoxime, aminoguanidine bicarbonate, bromoform, ter-butyl bromide, chloral hydrate, chloropierin, dimethylglyoxime, hexamethylenetetramine, iodoform, isobutylbromide, methylglyoxal sodium bisulphite, monochlorohydrine, oxamide, propionamide, sulphonal, thialdine, tribromohydrine, trimethylene bromide, trional, veronal.

BENZENE AND TOLUENE DERIVATIVES

No Color Reaction.—Azoxybenzene, ortho-bromochlorobenzene, para-bromochlorobenzene, meta-chloronitrobenzene, para-chloronitrobenzene, para-chlorotoluene, ortho-dichlorobenzene, 2, 5-dichloronitrobenzene, 2, 4-dinitrochlorobenzene, diphenyl, isopropylbenzene, meta-nitrotoluene, ortho-nitrotoluene, para-nitrotoluene, styrene.

ANILINE AND DERIVATIVES

Acetylphenetidine	Brown
Aminoazobenzene	Reddish brown in cold
meta-Anisidine	Brownish black
ortho-Anisidine	Brownish yellow
para-Anisidine	Yellow in cold
para-Benztoluide	Black
para-Chloroaniline	Yellow in cold
1, 2, 4-Dinitroaniline	Rose in cold, orange with heat
Diphenylamine	Reddish brown
Diphenyl benzidine	Red
ortho-Phenetidine	Reddish brown in cold
para-Phenetidine	Brown
ortho-Toluidine	Black
Tribromoaniline	Black
Trinitroaniline	Deep yellow

No Color Reaction.—Acetoanilide, acetphenetidine, acetyl-para-anisidine, acetyl-ortho-methyltoluidine, acetyl-para-methyltoluidine, meta-acetxylidine, 2-aminotoluene-4-sulphonic acid, 2-aminotoluene-5-sulphonic acid, 4-aminotoluene-2-sulphonic acid, aniline, benzanilide, benzidine, ortho-benztoluide, para-bromoacetanilide, meta-bromoaniline, ortho-bromoaniline, para-bromoaniline, chloramine, meta-chloroaniline, ortho-chloroaniline, 1, 2, 4-dichloroaniline, 2, 4-dichloroaniline, exalgine, meta-nitroaniline, para-nitroaniline, meta-nitrodimethylaniline, para-nitrodimethylaniline, nitrosodimethylaniline, 1, 2, 3-nitrotoluidine, 1, 2, 4-nitrotoluidine, 1, 3, 4-nitrotoluidine, phenyl-beta-diphenylamine, metatoluidine, paratoluidine, 1, 2, 4-xylidine, 1, 3, 4-xylidine.

PHENOLS AND DERIVATIVES

ortho-Aminophenol	Greenish black
5-Benzalamino-2-cresol	Black in cold
Bromothymol	Orange
ortho-Chloromercuriphenol	Rose, trace of violet
ortho-Cresol	Red
Orcinol	Dark brown
Resorcinol	Cherry red in cold
Thymol	Deep red
Xylenol	Brown

No Color Reaction.—Acetyl-meta-aminophenol, acetyl-para-aminophenol, meta-aminophenol, para-aminophenol, benzoylthymol, para-bromophenol, carvacrol, catechol, ortho-chlorophenol, para-chlorophenol, meta-cresol, 2, 4-dichlorophenol, dimethylhydroresorcinol, 2, 3-dinitrophenol, 2, 6-dinitrophenol, meta-nitrophenol, ortho-nitrophenol, para-nitrophenol, phenol, phloroglucinol, picric acid, pyrogallic acid, tetrabromo-ortho-phenol, tribromophenol, trichlorophenol.

AROMATIC ACIDS

Naphthionic acid	Brown
Tannic acid	Black
Terephthalic acid	Violet black

No Color Reaction.—Acetylsalicylic acid, meta-aminobenzoic acid, para-aminobenzoic acid, anisic acid, anthranilic acid, arsanilic acid, benzilic acid, meta-bromobenzoic acid, ortho-bromobenzoic acid, para-bromobenzoic acid, meta-chlorobenzoic acid, ortho-chlorobenzoic acid, para-chlorobenzoic acid, cinchophen, cinnamic acid, cumaric acid, diiodosalicylic acid, 3, 5-dinitrobenzoic acid, diphenylacetic acid, gallic acid, 5-iodosalicylic acid, mandelic acid, metanilic acid, meta-nitrobenzoic acid, ortho-nitrobenzoic acid, para-nitrobenzoic acid, salicylic acid, sulphosalicylic acid, ortho-toluic acid, para-toluic acid.

AROMATIC ACID DERIVATIVES

Neocinchophen (ethyl-6-methyl-2-phenylquinoline-4-carboxylate)	Brownish green

No Color Reaction.—Benzamide, benzyl benzoate, butyl benzoate, cumarin, ethyl benzoate, ethyl salicylate, isoamyl benzoate, methyl benzoate, methyl cinnamic ester, methyl salicylate, nicotinic acid nitrate, phenyl salicylate, phthalimide.

AROMATIC ALDEHYDES, ETHERS, ALCOHOLS, AND KETONES

Anisole	Rose, trace of violet
Benzalacetone	Deep yellow
Benzhydrol	Brown
Benzophenone	Brown
ortho-Chlorobenzaldehyde	Blood red
Guaiacol	Violet
ortho-Nitrobenzaldehyde	Reddish brown
Tetramethyldiamino-benzophenone	Brown
Vanillin	Dark brown

No Color Reaction.—Para-Aminoacetophenone, anisaldehyde, benzil, benzyl alcohol, 1, 2, 5-bromosalicylaldehyde, meta-methoxysalicylaldehyde, phthalic acid aldehyde, salicylaldehyde, salicylaldehyde methyl ether, saligenin, para-tolylaldehyde.

HETEROCYCLIC COMPOUNDS

Acridine	Light green
6-Nitroquinoline	Reddish brown
Quinoline	Dark green
Skatole	Green in cold, black with heat

No Color Reaction.—Antipyrine, isatin, oxyquinoline, piperidine, quinaldine.

HYDROAROMATIC COMPOUNDS

Carvenone	Reddish violet in cold
Menthol	Violet black
Terpenyl acetate	Brown

No Color Reaction.—*d*-Borneol, *l*-borneol, *dl*-camphor (natural), camphor (synthetic), camphoric acid, camphor-sulphonic acid, limonene, terpineol.

NAPHTHALENE AND ANTHRACENE DERIVATIVES

Acet-alpha-naphthalide	Brownish black
Acet-beta-naphthalide	Greenish brown
Alizarin	Reddish brown
Dibromoanthracene	Yellowish brown
beta-Naphthol	Brownish violet
alpha-Naphthylamine	Greenish brown
beta-Naphthylamine	Brown
alpha-Naphthylaminoazobenzene	Reddish brown

No Color Reaction.—Alpha-Bromonaphthalene, 1, 5-dinitronaphthalene, beta-naphthalenesulphonic acid, naphthalic anhydride, alpha-naphthylisocyanate.

MISCELLANEOUS AROMATIC COMPOUNDS

Abietic acid	Brownish violet
Dibromothymolsulphonphthalein	Reddish violet
Isoeugenole	Reddish violet
meta-Nitrobenzhydrazide	Brown
Phenanthrene	Violet black
meta-Phenylenediamine hydrochloride	Brown in cold
Phenylhydrazine hydrochloride	Orange
Salvarsan	Green
Thiosemicarbazide	Brownish orange
Thymolphthalein	Reddish lavender

No Color Reaction.—Adrenaline, amarine, benzcatechin, benzoin, benzylphenylhydrazine, eugenol, 2, 4-dinitrophenylhydrazine, para-nitrophenylhydrazine, phenanthrenequinone dioxime, phenolphthalein, quersite, rheumatine (saloquinine salicylate), tetrabromophenolphthalein, para-tolylisothiocyanate, triphenylmethane, turmeric.

The color reactions for the tests where an equal amount of impurity was added to the atropine were recorded, and also the reactions where five times the amount of impurity was added; but the listing of these would require too much space. Therefore, only those reactions in which the reddish violet color was completely masked will be given. The list of substances previously given may be referred to in order to obtain the names of the compounds which did not completely cover up the atropine test. Many of these compounds gave no interference, while others gave more or less interference.

Organic compounds which completely covered up the atropine test when present in equal amounts were: acetylsalicylic acid, aminoazobenzene, amyl

alcohol, antipyrine, azoxybenzene, benzalacetone, benzidine, para-bromophenol, bromothymol, *dl-n*-butylthiourea, ortho-chlorobenzaldehyde, colocynthin, dibromoanthracene, diphenylbenzidine, duleitol, elaterin, eugenol, glycine, guaiacol, isobutyl alcohol, morphine, alpha-naphthylamine, beta-naphthylamine, narcotine, ortho-nitrobenzaldehyde, orcinol, phloridzin, sanguinarine, skatole, sparteine, strychnine, thiobarbituric acid, para-toly-isothiocyanate.

The organic compounds, in addition to those listed above, which completely covered up the atropine test when present in amounts five times that of atropine were: acetyl-meta-aminophenol, acridine, allylphenylthiocarbamide, allylthiocarbamide, para-anisidine, 5-benzalaminophenol, benzhydrol, benzophenone, carvenone, para-chlorophenol, cryptopine, delphinine, 2, 4-dichlorophenol, 1, 2, 4-dinitroaniline, 1, 5-dinitronaphthalene, diphenylamine, edestine, melezitose, metanilic acid, naphthionic acid, metanitrobenzoic acid, para-toluidine, trinitroaniline, 1, 2, 4-xylidine, xylose.

From a study of the color reactions given in the tables, it will be observed that there were twenty-nine compounds which gave various shades of violet, lavender, or purple, either alone or combined with other colors. Many of these might have been mistaken for the atropine test. In general, the compounds giving a test similar to that of atropine do not belong to any definite group of organic compounds, nor does any special organic radical seem to be responsible for the characteristic test. The alkaloids as a class gave more positive reactions than any other group.

There were a few of the organic compounds which completely masked the atropine test. Others showed interference in varying degrees. Of course, in a carefully conducted analysis, many of these substances would be removed by means of the different organic solvents.

CONCLUSIONS

1. A number of organic compounds have been found which give a positive atropine test with para-dimethylaminobenzaldehyde reagent.

2. The interferences caused by organic compounds as impurities have been determined.

REFERENCES

1. Levine, V. E.: Studies in Toxicologic Chemistry, J. Lab. & Clin. Med. **11**: 809, 1926.
2. Levine, V. E., and Magiera, E. A.: Studies in Toxicologic Chemistry, J. Lab. & Clin. Med. **12**: 773, 1927.
3. Poe, C. F., and O'Day, D. W.: A Study of Mandelin's Test for Strychnine, J. Am. Pharm. Assn. **19**: 1292, 1930.
   Poe, C. F., and Stehley, Pearl S.: A Study of Frohde's Test for Morphine, J. Lab. & Clin. Med. **18**: 375, 1933.
   Poe, C. F., and Clemens, R.: A Study of Vitali's Test for Atropine, J. Lab. & Clin. Med. **18**: 743, 1933.
4. Wasicky, R.: A Very Sensitive Color Reaction for Atropine, Hyoscyamine, and Scopolamine, Ztschr. Anal. Chem. **54**: 393, 1915.
5. Ekkert, L.: Reactions of Atropine and Certain Related Compounds, Pharm. Ztschr. **71**: 180, 550, 1930. See Chem. Abs. **24**: 3081, 1930.

THE SIGNIFICANCE OF MEMBRANES IN THE MUCOUS FORM OF COLITIS*

JULIUS FRIEDENWALD, M.D., AND SAMUEL MORRISON, M.D., BALTIMORE, MD.

INTRODUCTION.—In a recent paper Boas[1] discusses colitis membranacea (or colica mucosa) as a disappearing disease and compares its incidence thirty years ago with its rarity today. This is attributed to the therapeutic measures employed in the treatment of the simple forms of colitis. Modern methods of treatment no longer include medicated and astringent enemas as important aids in combating colitis, and in Boas' opinion, the frequency of the disease has diminished in proportion to this change. Boas has found that in his recent cases of membranous colitis a history of habitual use of enemas has always been elicited. Interestingly enough the substitution of dietary treatment in place of frequent utilization of enemas was followed by the disappearance of membrane formation. It seems therefore quite clear, according to him, that membranous colitis is the result of radical treatment of simple forms of colitis with enemas and cathartics.

McCrae[2] speaks of mucous colitis as a remarkable disease and gives as its synonyms such names as membranous enteritis, tubular diarrhea, mucous colic, and myxoneurosis intestinalis. It is a disease which has been recognized for many centuries. But a distinction must be made between the colitides associated with catarrh of the intestines and due to innumerable causes, and those due to local disease in the bowel as well as those associated with true mucous colitis. The latter is usually considered a *secretory neurosis* of the large intestine and is encountered principally in those patients (females more frequently than males) who have unstable nervous mechanisms. However, though some cases may be primarily psychogenic in origin and others organic, the majority have present both organic and psychogenic dysfunctions and even in those instances in which one factor is very much more prominent the other is doubtless contributory.

Boas[1] presents his views on the nature of mucous (membranous) colitis and calls attention to the many factors which may enter into its etiology. Briefly, he believes the disease may be dependent upon a primary affection of the colon in the nature of an inflammatory condition or that it may result from a secondary affection based upon a neuropathic, vagotonic state of irritation. Since the causative agent is so doubtful, it is but little wonder that the therapeutic measures employed in these cases should be at such variance. In Boas' experience, an experience which he has reason to believe is general, the disease was much more common in the latter part of the nineteenth century than it has been in the past decade. So much is this so that very little has been published

*From the Gastro-Enterological Clinic of the Department of Medicine of the University of Maryland.

Received for publication, October 20, 1932.

upon this subject in the past ten years, and thus this author makes inquiry concerning the reasons for the loss of interest in this affection.

In referring to the etiology of colitis membranacea, Boas believes there is definite evidence to indicate that the disease syndrome is due to a superficial catarrh involving especially the lower intestinal segment. However, the important rôle played by a disharmony in the vegetative nervous system is recognized. Nevertheless, certain facts are apparent when the superficial colitis itself is considered, and as Boas had previously pointed out, astringent substances in the form of enemas, so commonly used in the past, undoubtedly favor the formation of membranes. For example he had called attention to the fact that in patients suffering from the usual form of mucous colitis, small tannin enemas given successively in the course of a few days usually led to the formation and to the discharge of typical membranes which were previously absent; with the cessation of treatment, the formation of membranes disappeared in a short time, to reappear with the institution of astringent enemas.

Furthermore, Boas performed the following interesting experiment: Intestinal mucus was placed in a 1 to 2 per cent tannin solution and in the course of twenty-four hours a definite brown color and a membranous appearance were observed which became more pronounced in the course of the subsequent twenty-four hours. The membranes formed here did not differ histologically from the typical membrane formation in colitis membranacea or colica mucosa. Similar results were obtained when the intestinal mucus was treated with other astringent substances such as lead acetate. It is even conceivable, Boas remarks, that the addition of such harmless substances as borax, glycerin, or soap, to the enema, or even the repeated use of water enemas of an unsuitable temperature, may give rise to a catarrhal inflammation of the colon which is followed by an increased production of mucus and finally by the formation of artificial membranes.

Recently, Friedenwald and Feldman[3] reported upon the effect of the prolonged use of colon enemas in animals, because it was their experience that the all too frequent use of enemas and irrigations led to the production of certain colonic affections, as well as nervous intestinal disorders. In a well-controlled series of animal experiments they administered plain water, soap water, cotton seed oil, sodium carbonate solution, neutral acriflavine solution, camphor water, ammonium chloride solution, and hydrochloric acid. They concluded that enemas used over a long period of time led to more or less bowel disturbance, that even simple water enemas may produce mild inflammatory changes, that soapsuds enemas produce more marked inflammatory changes while oil enemas produce less irritation, that acriflavine (1:3000) and camphor water (U.S.P.) cause but slight disturbance of the bowel and that irritating chemicals such as sodium carbonate, ammonium chloride, and hydrochloric acid, even in weak solutions cause inflammatory changes ranging from mild processes to extensive ulcerations and necroses.

Similar observations have been made by Hirschman,[4] and Runge and Hartman.[5] Even though these findings, obtained mostly in animal experimentation, cannot be applied directly to man, it is still clear, in Boas' opinion, that the use of certain enemas, which were very popular a decade ago in the treatment of

various affections of the colon, actually favored the appearance of catarrhal changes and ulcers. Since astringent enemas are not habitually used today the reason for "colitis membranacea, a disappearing disease" becomes apparent and this disappearance is further accounted for by the fact that the irritative cathartics have been substituted by nonirritating substances.

Thus, there is established a connecting link between the so-called simple colitides and the membranous type. The question therefore arises concerning the inherent value of enemas and irrigations, and in the discussion of this problem, the views of various observers are by no means in accord. In Soper's[6] experience "the more one irrigates, the more mucus one gets. In other words, irrigations incite the secretion of mucus and if continued long enough, the 'foul smelling material' can be secured in persons with normal colons. The material is, in fact, the normal contents of the ileum when subjected to such treatment." On the other hand Bastedo[7] finds that "the slow, careful introduction of from six to ten gallons of water is a valuable therapeutic measure in the treatment of mucous colitis. . . ." In his conclusion, Soper remarks that "mucous colitis has been set up as an obscure and often incurable disease. It is neither an inflammatory process nor a neurosis but is a spasm of the colon in which long strings of mucus are passed. The colon is an instrument that is played on by many and varied outside influences. I may mention the psychoses, gallstones, kidney stones, ulcer of the stomach and duodenum, pelvic diseases, and allergy. I have made roentgenograms showing colon spasm in the conditions mentioned. Purgative drugs, enemas, and irrigations all produce further irritation and more tendency to spasm. The underlying cause should be eradicated if possible. If the condition is not to be diagnosticated, treatment should consist of retention enemas of liquid petrolatum, a smooth diet, and mild sedatives, with avoidance of all colonic manipulation."

Experiments.—In view of Boas' work a series of experiments was planned in order to confirm or repudiate some of the opinions already briefly summarized.

First, we obtained mucus in its various forms (shreds, strings, and membranes) from some of our mucous (or mucomembranous) colitis cases, and we conducted a series of "testtube" experiments. For example, we placed bits of mucus of definite size in a bottle containing 100 c.c. of water. This was utilized as a control. In another bottle the same sized portions of mucus were placed but its medium was 100 c.c. of 2 per cent tannin solution. This experiment was repeated frequently with the following results. In twenty-four hours the water bottle showed little or no change except, perhaps, for a slight thinning of the mucus, but this may have been more apparent than real; the tannin bottle, on the other hand, seemed to contain more mucus than was originally added, but there was no increase in membrane formation. How the increased cloudiness of the tannin solution itself was to be evaluated presented some difficulty but we were well able to measure an increase in mucous surface. In forty-eight hours both solutions had become cloudy; the tannin bottle was obviously the cloudier. In these latter observations it was noted that many of the mucous particles had disintegrated, and this doubtless accounted for some of the turbidity of the solutions.

The same experiments were repeated using in place of the shreds and strings of mucus large thin membranes obtained from a patient who was under our care at the time. The membranes were placed in solutions of water, 2 per cent tannin and soapsuds. Twenty-four hours later very little change was noted in the membranes which measured two inches square. Forty-eight hours later we were still unable to detect any change and for the next two days the observations were essentially negative. However, after a week it was noted that the sheets of mucus had become stiffer and thicker in the tannin medium as compared with the water medium in which they remained thin and for the most part, unchanged. A curious change takes place in the soapsuds medium where some sort of reaction between the soap and mucus results in a thick gelatinous solution but without any distinct change in the membrane of mucus itself.

It seemed, therefore, that whereas tannin solution (2 per cent) did affect mucous shreds and strings so that the amount of mucous substance seemed increased, it had little or no effect on membranes, nor did it seem to form membranes even after exposure of the mucus to such a solution for a period extending over a week. The results were practically negative with water solutions and equivocal or indecisive with soapsuds solution. If 2 per cent tannin had so little effect on membranes after seven days, it seemed difficult to understand the objection to its use as a therapeutic agent. It was thought, however, that the repeated use of freshly prepared 2 per cent tannin solution might cause more definite changes in membrane formation; when this was carried out the results were not altogether conclusive. The mucus assumed a brown color but the membranous appearance did not become more pronounced. It was suggested, therefore, that a 10 per cent solution of tannin be utilized.

Preparations were made of thin films of mucus in 10 per cent tannin solution and as a control in water. After twenty-four hours the tannin solution was found to contain a definitely thicker and tougher piece of mucus than in the water preparation. The preparations were allowed to stand for five days and though daily the difference already described was always distinctly noted, it was found that as time passed even the mucus in the plain water assumed similar, though not as pronounced, characteristics. After ten days the mucus in 10 per cent tannin was not very hard, but it was definitely coagulated while that in plain water had become soft and was easily macerated by light pressure.

Preparations of mucus in normal salt solution, 2 per cent tannin and plain water were made. Twenty-four hours later the tannin preparation was found to contain what may be described as a more durable mucus which was not increased in quantity. No change was noted in either of the remaining two media. After a week, coagulation of mucus had occurred in the tannin solution, much less marked in the salt solutions and not at all in the plain water. The same experiment was carried out with 2 per cent tannin, soap solution and plain water and the changes in the tannin and water preparations were exactly similar to those just noted. On the other hand the mucus in the soap solution seemed to have increased both in quantity and thickness (gelatinous). Daily observation over a period of five days disclosed several interesting findings. Mucus underwent gradual coagulation in the 2 per cent tannin solution; it became

tougher, thicker, and slightly shrunken. In the soap solution it assumed an apparent increased thickness due partly, it was later discovered, to the adherence of soap to the mucus. Even in plain water, mucus became a more thickly gelatinous substance.

Other solutions were used. Preparations of mucus in water, 2 per cent protargol, 1 per cent silver nitrate, 1:10,000 potassium permanganate, 1:3,000 neutral acriflavine, and ½ per cent sodium bicarbonate. These solutions were chosen because they are among those frequently used as enemas and irrigations in cases of colitis. After twenty-four hours it was noted, in brief, that the mucoid shreds were thinner in the water medium, somewhat thicker, and coagulated in the protargol and silver nitrate solutions, equally coagulated but slightly more resistant and indurated in the potassium permanganate solution, markedly coagulated and contracted in the neutral acriflavine and hardly changed in the sodium bicarbonate solution. After forty-eight hours the changes were essentially the same except for the neutral acriflavine solution in which the mucus had not only become markedly contracted but had been dissolved so that very little remained. Four days later the mucus (shreds and membrane) in the plain water had become thinner in the protargol, discoloration had occurred and coagulation with contraction had become more obvious; essentially the same changes took place in the silver nitrate solution as had resulted in the protargol preparation, discoloration was noted in the potassium permanganate preparation while induration and coagulation had progressed; in neutral acriflavine the changes were as already noted except that dissolution of mucus had not apparently continued; little or no change was noted in the bicarbonate preparation. After five days, observations were made on the silver nitrate and neutral acriflavine preparations. The former disclosed a considerably thickened mucus while in the latter the mucus had shrunken remarkably. After seven days the mucus in the silver nitrate solution had undergone further contraction in addition to assuming a black color. In the neutral acriflavine solution the mucus had contracted into a small hard mass but in addition the preparation contained many disintegrated bits of mucus resembling ordinary débris. The preparations were allowed to stand for six more days and during that time the mucus in the plain water preparation had become thinner but was otherwise unchanged. In the protargol solution induration, coagulation and some increase in quantity were noted. The silver nitrate preparation contained a hardened contracted mass of black material no longer resembling mucus. Hardly any further change was observed in the potassium permanganate solution except for an additional slight increase in the thickness of the membranes. The neutral acriflavine presented a picture unlike any of the other solutions. In this instance, the mucus was thickened, contracted, "gummed," granular and in parts apparently dissolved. The bicarbonate solution had not produced any change beyond a slight degree of coagulation.

These experiments, then, sum up our observations on the effect of various astringent (as well as nonastringent) solutions upon mucus, in vitro. Our next problem was to employ the same agents in vivo and to this end we produced changes resembling colitis in dogs by administering enemas of irritating solutions

such as hydrochloric acid (½ to 10 per cent) and tannin (10 to 20 per cent). When the stools became diarrheic and contained an occasional trace of blood, enemas of various astringent solutions were injected.

Two dogs were given (75 c.c.) enemas of 10 per cent tannin solution three times a day. The stools were carefully observed. After one week had passed neither mucus nor diarrhea appeared. A 15 per cent solution of tannin was prepared and later a 20 per cent solution, but we were unable to produce either diarrhea or mucus. This experiment was abandoned. The same animals were then given 90 c.c. of 10 per cent hydrochloric acid per rectum once daily for two days. One of the dogs reacted by refusing his food ration and losing weight. He quickly became moribund and practically helpless and did not avoid soiled parts of his cage. The other dog, although weakened, was relatively in good health. The 10 per cent hydrochloric acid enemas were discontinued and the tannin enemas (60 to 90 c.c. twice daily) resumed.

After the tannin enemas had been administered for two days following the discontinuance of the 10 per cent hydrochloric acid, the sickly dog previously mentioned died. At autopsy, the entire lower bowel was found to be gangrenous, and there was a large perforation in the cecal region. The most interesting finding was concerned with the mucoid coating of the entire lower bowel. The mucus could not be peeled away in the form of membranes nor was it easy to separate it satisfactorily in any definite form except thin shreds. It adhered closely, in all its thickness, to the mucosa of the intestine. One could easily picture its possible separation as a membrane or mold of the intestine if appropriate measures were employed.

In the meantime gross and occult blood had appeared in the stools of the remaining dog and diarrhea had occurred. This was allowed to continue for four days and in the meanwhile tannin enemas were administered. They were then discontinued and for them were substituted enemas of 1 per cent hydrochloric acid (60 c.c. t. i. d.). The stools became less numerous, the mucus disappeared as well as the blood. Tannin enemas and other astringent solutions were used but the results were so indefinite that it was decided to sacrifice the dog after two more weeks of such experimentation. The dog had apparently recovered completely. He was sacrificed and at autopsy the lower bowel (rectum and sigmoid colon) was found to be congested, injected, indurated, and inflamed. At the sigmoid rectal junction and for two or more centimeters below this region (almost extending to the anus in places), the mucous membrane had become so indurated that it resembled cartilage. Coincidentally two round worms (5 cm. in length) were found around the appendix in one area of which the wall had become so thin as to be on the verge of perforation. Strikingly enough the entire large intestine was thickly coated with mucus, typical in appearance but difficult to separate from the mucous membrane. No ulcerated areas were found in the intestines.

In the human being, the production of excessive mucus after treatment with astringent solution has long been noted. Nevertheless, the possibility that these quantities of mucus could be the result of treatment hardly seemed to arouse serious consideration, except occasionally, until some of the more recent

reports, of which that of Boas is an example. However, long before this, many physicians had, through experience, begun to diminish the frequent use of irrigations and enemas in such conditions. Even in cases of ulcerative colitis, it was concluded by many observers that enemas often caused complications and may also have been responsible, at times, for the poor results obtained. At any rate, their use in mucomembranous colitis (comprising that entire group of mucous and membranous colitides) was doubtless more irritative than curative and seemed to favor the formation of membranes.

In *summary*, therefore, we have observed the effect of certain solutions upon mucus in vitro and in vivo, and we have been able to demonstrate that these solutions certainly bring about abnormal changes in mucous strings, shreds, and membranes in vitro. We have, however, not been able to demonstrate membrane formation with any degree of constancy in these vitro experiments. Our results confirm in many respects, the observations made by Boas. On the other hand, our vivo experiments on dogs have led us to believe that membranes are formed, but that they do not separate with ease from the intestinal wall.

Conclusions.—As the result of our observations it is evident (1) that many solutions, and particularly those of an astringent nature, undoubtedly are irritative to the intestinal mucosa and may cause changes in the form of mucus, *sometimes* producing actual membrane formation. (2) Bowel irrigations and medicated enemas should therefore be utilized with the greatest caution; they should be administered only at infrequent intervals and the solutions used should always be well diluted.

REFERENCES

1. Boas, I.: Colitis Membranacea, A Disappearing Disease, Med. Klin. **27**: 1451, 1931.
2. McCrae, T.: Osler's Principles and Practice of Medicine, 1927, New York and London, D. Appleton & Co., p. 548.
3. Friedenwald, J., and Feldman, M.: Experimental Studies on the Effect of the Prolonged Use of Colon Enemas Upon the Bowel in Animals, Am. J. Surg. **11**: 23, 1931.
4. Hirschman, L. J.: Enemas: Some of Their Uses and Abuses, J. A. M. A. **89**: 1039, 1927.
5. Runge, H., and Hartman, H.: Clinical and Experimental Investigations on Injuries of the Intestine Following Soap Enemas, Klin. Wehnschr. **7**: 2389, 1928.
6. Soper, H. W.: Colon Irrigations, J. A. M. A. **98**: 1677, 1932.
7. Bastedo, W. A.: See Reference 6.

SOME FACTORS RESPONSIBLE FOR THE SO-CALLED SELF-DISINFECTING POWER OF THE SKIN*†

CLAUDE S. BRYAN, M.S., AND W. L. MALLMANN, PH.D., LANSING, MICH.

NO SATISFACTORY explanation has been presented for the fact that bacteria die rapidly when smeared upon the surface of the skin. That pure cultures do die at a remarkably high rate was first demonstrated by Arnold and his coworkers[1] who showed that reductions of 90 to 100 per cent could be effected in ten to thirty minutes. They demonstrated that the condition of the skin apparently caused marked changes in the rate of reduction, as evidenced by the fact that a clean skin caused a more rapid killing action than a dirty skin. Cornbleet and Montgomery,[2] in a series of studies on normal and pathologic skins, demonstrated variable results, using yeast and *Staphylococcus aureus* as their organisms. They found that moist areas were less effective in destroying the test organisms than dry areas, that denuded areas were less effective than normal areas, and that, in the case of psoriasis, lesions freed of scales were more active than those with scales. These data would strongly indicate that desiccation played an important rôle, as in each instance cited above, the most successful results were obtained under conditions where desiccation was the most rapid. Systemic changes induced by the injection of gentian violet and manganese butyrate also caused changes in the rate of destruction. A depression occurred after the immediate injections, followed later by increased rates, higher than normal. No explanation is offered for the latter results. Norton and Novy[3] in a series of experiments using *Bacillus prodigiosus* found that bacteria dried on inert materials died at approximately the same rate as on the normal skin. They conclude that the living skin has no inherent germicidal power. Fisher[4] demonstrated decided fluctuations in the rate of destruction during menstrual periods when a depressed action occurred. An examination of her data shows variability from day to day and, in one instance, marked depressions at times outside the menstrual period. These marked daily fluctuations in rate of destruction suggested the work presented in this paper.

PROCEDURE

A scientific technic for obtaining comparable results for studies of the viability of bacteria placed upon the surface of the skin or upon inert materials appears to be very difficult. No matter how the organisms are introduced upon the surface or how they are removed for examination, directly comparable results are not obtained. It seems quite reasonable to suppose that bacteria dried on a smooth surface, like glass, could be removed more readily than from an irregular surface like living skin. Furthermore, the time elapsing between smearing and

*From the Department of Bacteriology, Michigan State College.
Received for publication October 20, 1932.
†Journal Article No. 112 (n. s.) from the Michigan Agricultural Experiment Station.

removal of the bacteria would change the degree of attachment of the bacteria to the surface, due to the fact that the process of drying causes a closer adherence of the bacteria to the surface. Thus the longer the period of contact the greater the difficulty of removal. Granting a uniform procedure of removal, the longer the interval of exposure, the fewer organisms would be obtained. However, since similar results were always obtained under similar conditions, the data are of comparative value in measuring the effectiveness of the various procedures used. The writers deplore the lack of a more satisfactory means of measurement.

The procedure was essentially the same as that used by others. All test organisms were twenty-four-hour broth cultures. Each culture was thoroughly shaken to give an even suspension before use. An appropriate amount of the broth culture was added to sterile saline in order to obtain the proper number of bacteria. These dilutions were always prepared just prior to performing the experiments and in every case where more than thirty minutes elapsed between trials, a fresh dilution was prepared to avoid the effect of killing the organisms in the salt solution.

For convenience, the back of the hand was used for the skin tests in all cases. The hands were thoroughly washed before each experiment. Seven areas of 9 sq. cm. each were marked off with a red wax pencil. Exposures of five-tenths, one, two, three, five, ten, and fifteen minutes were used. Smears were made with a sterile cotton swab immersed in the dilution of the culture. After the proper exposure, the surviving bacteria were removed by swabbing the surface with a sterile cotton swab, previously dipped into a sterile dilution blank. The swab was then immersed in sterile saline dilution blanks of 100 c.c. amounts and shaken thoroughly. The length of time, the vigor of the swabbing, and the thoroughness of the rinsing of bacteria from the swabs were standardized in all instances to eliminate as far as possible errors due to technic. Immediately after preparing the dilutions, dextrose beef extract agar dilution plates were made. All counts were made after twenty-four hours' incubation at 37° C. *Staph. aureus, Serr. marcescens,* and *Escherichia coli* were used as test organisms.

EXPERIMENTAL

Norton and Novy[3] state that no inherent germicidal power was obtained for living skin over inert materials. Apparently, in the experiments presented these tests on skin and inert materials were made at different times with different subcultures as a source of the test organisms. To determine the significance of conducting the tests on inert material and skin simultaneously, cultures of *Esch. coli* and *Staph. aureus* were smeared on clean skin, glass, dry filter paper and moist filter paper at the same time using the same culture dilution. The tests were conducted at room temperature. Due to the fact that the bacteria frequently failed to survive an exposure of ten minutes on either inert materials or skin, intervals of five-tenths, one, two, three, five, ten, and fifteen minutes were used. Sterility, as measured by the absence of colonies on the agar plates, was obtained first on the skin, followed by dry filter paper, dry glass, and moist filter paper respectively. These data, which are representative of repeated tests, indicate that under these conditions the skin causes a more rapid destruction of the bacteria.

It may be argued that, the temperature of the skin being higher than that of the glass and filter paper, desiccation would be more rapid in the former case and that if the killing action is due to desiccation, the skin would show a greater effect. To determine this a series of experiments was conducted at 37° C. so that the temperatures of all surfaces would be approximately the same. The data for *Esch. coli* are presented in Table I. It will be noted that similar results were obtained. The difference in killing action between the skin and the glass, however, is not as great, indicating that desiccation does play an important part. Similar results were obtained with *Staph. aureus* and *Serr. marcescens*, as shown

TABLE I

THE INFLUENCE OF DESICCATION ON ESCHERICHIA COLI AT 37° C.

TIME OF EXPOSURE	SUBSTANCE TESTED					
	SKIN		GLASS		FILTER PAPER MOIST	
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
30 seconds	78,000		107,000	0	251,000	
1 min.	77,000	1.29	107,000	0	201,000	9.93
2 min.	53,000	32.06	78,000	27.11	198,000	21.12
3 min.	51,000	34.62	69,000	35.52	191,000	23.91
5 min.	800	98.98	44,000	58.88	188,000	25.10
10 min.	0	100.	500	95.33	168,000	33.07
15 min.	0	100.	0	100.	149,000	40.64

TABLE II

THE INFLUENCE OF DESICCATION ON STAPHYLOCOCCUS AUREUS AT 37° C.

TIME OF EXPOSURE	SUBSTANCE TESTED					
	SKIN		GLASS		FILTER PAPER MOIST	
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
30 seconds	161,000		225,000		173,000	
1 min.	133,000	17.5	230,000	–2	175,000	– 1
2 min.	92,000	42.9	130,000	42.3	182,000	– 4
3 min.	112,000	30.5	103,000	58.7	232,000	–32
5 min.	40,000	75.2	122,000	45.8	180,000	– 2
10 min.	37,000	77.1	97,000	56.9	168,000	2.9
15 min.	34,000	78.9	97,000	56.9	170,000	1.9

TABLE III

THE INFLUENCE OF DESICCATION ON SERRATIA MARCESCENS AT 37° C.

TIME OF EXPOSURE IN MINUTES	SUBSTANCE TESTED					
	SKIN		GLASS		FILTER PAPER MOIST	
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
½	270,000		110,000		510,000	
1	254,000	5.93	104,000	5.46	375,000	26.87
2	16,000	94.08	122,000	–10	354,000	30.59
3	17,000	93.71	111,000	–0.9	223,000	56.28
5	6,000	97.78	95,000	13.64	165,000	67.65
10	0	100.	0	100.	155,000	69.61
15	0	100.	0	100.	141,000	72.36

in Tables II and III respectively. The data presented thus indicate that living skin does cause a more rapid destruction of bacteria than inert materials under similar conditions.

The fact that the same individual on repeated tests on successive days gives variable results may not necessarily be due to variations in technic. Marked variations do occur as demonstrated by Fisher[4] in her studies on the influence of the menstrual cycle. Fluctuations also occur in normal individuals from day to day. These variations are likely due to local or systemic changes of the body. Walker and Pryer[5] demonstrated that water irradiated with ultraviolet light had imparted to it through the exposure a residual germicidal power that was evident for several hours. An attempt was made to see if such residual germicidal powers could be produced in the skin. Accordingly, the hands of three individuals were tested for self-disinfecting power using *Serr. marcescens* and then the same hands were irradiated with ultraviolet light for one minute.

TABLE IV

INFLUENCE OF IRRADIATION BY ULTRAVIOLET LIGHT ON THE SELF-DISINFECTION OF THE SKIN USING SERRATIA MARCESCENS AS A TEST ORGANISM

TIME OF EXPOSURE IN MINUTES	SUBSTANCES TESTED							
	SKIN						GLASS	
	PERSON A		PERSON B		PERSON C			
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
Before Irradiation								
½	224,000	0.	279,000	0.	218,000	0.	200,000	0.
1	212,000	16.	286,000	0.	235,000	0.	191,000	5.
2	192,000	15.	215,000	25.	181,000	28.	179,000	11.
5	58,000	74.	81,000	71.	74,000	68.	168,000	17.
10	38,000	82.	38,000	85.4	32,000	87.2	159,000	22.
15	32,000	86.2	32,000	89.	36,000	85.4	134,000	38.
After Irradiation								
½	273,000	0.	261,000	0.	238,000	0.	254,000	0.
1	232,000	14.	254,000	4.	241,000	0.	229,000	9.8
2	229,000	16.	238,000	8.	219,000	13.	241,000	5.1
5	10,000	96.3	13,000	50.	14,000	42.	184,000	27.5
10	0	100.	0	100.	0	94.6	152,000	37.6
15	0	100.	0	100.	0	100.	141,000	44.5

Approximately five minutes after the exposure the same hands were tested for self-disinfecting power, using the same subculture of *Serr. marcescens.* The data are presented in Table IV. At the end of fifteen minutes' exposure of the organisms to the skin, prior to exposure to ultraviolet light, only 85 to 89 per cent of the bacteria were destroyed; whereas sterility was obtained in ten minutes after exposure to ultraviolet light. At the same time controls on glass showed only a reduction of 38 to 44.5 per cent. Repeated experiments on these same individuals and others gave similar results. Similar tests were made on chickens. The data for one such experiment are presented in Table V. The results are the same except that the difference between the counts obtained before and after irradiation was less marked. The higher body temperature of the chicken may

TABLE V

INFLUENCE OF ULTRAVIOLET LIGHT UPON SELF-DISINFECTION OF THE SKIN OF THE CHICKEN USING SERRATIA MARCESCENS AS A TEST ORGANISM

TIME OF EXPOSURE	GLASS		SKIN OF CHICKEN			
			BEFORE IRRADIATION		AFTER IRRADIATION	
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
½ min.	83,000		95,000		64,000	
1	76,000	8.44	73,000	21.06	15,000	76.41
3	75,000	9.65	32,000	66.32	13,000	79.69
5	75,000	9.65	15,000	84.22	10,000	84.38
10	64,000	22.90	5,000	94.74	0	100.
15	65,000	21.69	0	100.	0	100.

have caused a more rapid evaporation of the moisture with the result that desiccation played a greater rôle and thus a less significant difference, caused by the residual action of the ultraviolet light, would be obtained.

In the experiments cited, the action of the ultraviolet light was apparently a local residual effect. To determine whether or not the exposure to ultraviolet light would impart to the body an increased systemic self-disinfecting power, one hand was irradiated while the other was protected against exposure. Approximately five minutes later both hands were tested for self-disinfecting power using *Staph. aureus* as a test organism. This organism was used because Cornbleet and Montgomery[2] failed to find that exposure to ultraviolet light had any significance as far as self-disinfecting power of the skin was concerned. The

TABLE VI

INFLUENCE OF ULTRAVIOLET LIGHT UPON SELF-DISINFECTION OF SKIN UPON PART OF BODY NOT EXPOSED TO THE DIRECT RAYS OF LIGHT USING STAPHYLOCOCCUS AUREUS AS A TEST ORGANISM

TIME OF EXPOSURE IN MINUTES	SURFACE TESTED							
	GLASS		HAND BEFORE IRRADIATION		HANDS AFTER IRRADIATION			
					HAND IRRADIATED		HAND NOT IRRADIATED	
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
½	371,000		368,000					
1	371,000	0	362,000	1.63	143,000	0	232,000	
2	369,000	0.57	349,000	5.17	146,000	0	206,000	11.22
3	367,000	1.08	299,000	18.75	144,000	0	204,000	12.07
5	367,000	1.08	284,000	22.83	75,000	47.84	204,000	12.07
10	370,000	0.27	286,000	22.29	76,000	47.14	102,000	56.04
15	368,000	0.81	203,000	44.85	6,000	96.51	53,000	55.17

data are presented in Table VI. In fifteen minutes, the skin before irradiation showed a reduction of 44.65 per cent; whereas the irradiated skin showed a reduction of 96.51 per cent. At the same time, the skin not directly irradiated showed a reduction of 55.17 per cent, an increased reduction of 10.32 per cent over the skin before irradiation. These experiments were repeatedly confirmed on the same individuals and others. Apparently as judged by these data,

exposure to ultraviolet light not only causes an increased activity locally, but also imparts a systemic response.

Undoubtedly the exposure to the ultraviolet light caused a reduction, as evidenced by the data; however, the mechanism of action is not known. It apparently is not due to a change of evaporation of the skin induced by the ultraviolet light exposure because the hand that was not irradiated also showed a reduction, provided the other hand had been exposed. The temperature of the light had no effect as control tests made under the same conditions without exposure to ultraviolet light caused no reduction.

Since exposure to the action of ultraviolet light imparted an increased germicidal activity to the skin, the value of sunlight was tested. These experiments were conducted in a manner similar to those cited. The hand tested was exposed to the sun by holding out of the window for fifteen minutes. In all of the experiments, the person stayed indoors prior to conducting the experiment

TABLE VII

INFLUENCE OF SUNLIGHT UPON SELF-DISINFECTION OF THE SKIN USING SERRATIA MARCESCENS AS A TEST ORGANISM

TIME OF EXPOSURE IN MINUTES	GLASS		SKIN			
			BEFORE EXPOSURE		AFTER EXPOSURE	
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
½	31,000	0	34,000		51,000	
1	31,000	0	32,000	5.8	29,000	43.2
2	31,000	0	32,000	5.8	29,000	43.2
3	31,000	0	33,000	2.9	21,000	58.7
5	32,000	0	32,000	5.8	6,000	88.3
10	34,000	0	800	76.5	0	100
15	30,000	3.2	0	100	0	100

to avoid previous contact with sunlight. The data are presented in Table VII. Examination of the data shows that sunlight gave results similar to those obtained with ultraviolet light.

The value of conducting the tests simultaneously is strikingly demonstrated by comparing the fluctuations in killing rates on glass in the different tables. Comparing the same organisms on different days, very marked variations were obtained. Such variables as humidity, amount of inoculum and viability of the organisms are very difficult to control, except when all the tests are made simultaneously.

CONCLUSIONS

1. Desiccation plays an important rôle in the self-disinfection of the skin.

2. Living skin has an inherent ability to destroy bacteria implanted upon the surface.

3. A residual germicidal action is imparted to the skin by irradiation with ultraviolet light. Both a local and systemic reaction is obtained. The local action is more intense than the systemic action.

4. Exposure to sunlight causes an increased killing action similar to that obtained by irradiation by ultraviolet light.

REFERENCES

1. Arnold, L., Gustafson, C. J., Bull, Thos. G., Montgomery, B. E., and Charlotte, Singer: The Self-Disinfecting Power of the Skin as a Defense Against Microbic Invasion, Am. J. Hyg. **2**: 345, 1930.
2. Cornbleet, Theo., and Montgomery, B. E.: Self-Sterilization of the Skin, Arch. Dermat. & Syph. **23**: 908, 1931.
3. Norton, J. F., and Novy, Marguerite: Studies on the Self-Disinfecting Power of the Skin, Am. J. Pub. Health **21**: 1117, 1931.
4. Fisher, Virginia: Variations in Self-Disinfecting Power of the Skin During the Menstrual Cycle, Proc. Soc. Exper. Biol. & Med. **25**: 952, 1931.
5. Walker, W. F., and Pryer, R. W.: Bactericidal Action of Water Treated by Ultra Violet Rays, Am. J. Pub. Health **11**: 703, 1921.

THE POSSIBILITY OF A PRESSOR PRINCIPLE IN THE BLOOD OF PERSONS WITH HYPERTENSION*

An Experimental Study

Albert H. Elliot, M.D., and Franklin R. Nuzum, M.D.,
Santa Barbara, Calif.

THE elevation of blood pressure in arterial hypertension is due to increase of peripheral resistance in the arteriolar bed. Pathologic alteration of the arterioles sufficient in extent and degree to cause mechanically an increased resistance is not found at necropsy in patients dying of hypertension.[1] Spasm of the arterioles, or better, increased tonus of the arteriolar walls, which varies in degree and intensity and probably precedes structural changes, is a conception which satisfactorily explains the disturbance of the circulation in this disease.

Increased arteriolar tonus could conceivably be brought about in one or both of two ways, namely, by vasoconstrictor influences mediated through the vegetative nervous system, or by the direct action of a substance circulating in the blood stream upon the smooth muscle cells of the vessel wall or the nerve endings about them. The first hypothesis lends itself with difficulty to experimental study. With the second hypothesis in mind, investigators have repeatedly attempted to demonstrate a vasoconstrictor activity of blood or its constituents obtained from patients with hypertension. Attention has been focused upon the possibility of an hyperadrenalinemia, as suggested by Vaquez. Various biologic tests have been studied which are supposedly specific for and very sensitive to adrenalin. The positive results obtained by early workers were disproved by O'Connor[2] and by Hülse,[3] who showed that the tests are not specific and that the vasoconstrictor activity of serum may depend upon the process of clotting. However, Hülse demonstrated a "sensitizing" effect of serum obtained from patients with chronic glomerulonephritis upon the blood pressure response of the experimental animal to adrenalin. The serum of

*From the Laboratories of the Santa Barbara Cottage Hospital.
Received for publication, October 13, 1932.

patients with "primary" hypertension had no such effect. Recently Kahlson and Werz[4] using the rabbit ear perfusion technic, concluded that fresh blood from patients with thyrotoxicosis, fevers, and hypertension contains a vasoconstrictor principle which is not present in the blood of normal persons. In their experiments the factors of viscosity, temperature, and the possible sensitizing effects of serum were carefully controlled. These workers were not dealing with a constrictor substance the result of clotting because citrated whole blood perfused within three minutes of drawing was used. Their results are of much interest but should be corroborated.

Another method of approach to the problem is to determine the blood pressure response of the experimental animal to the injection of whole blood or serum obtained from patients with hypertension. This has received comparatively little study and the results are conflicting. Stewart and Cushing[5] mentioned injecting 5 to 10 c.c. of such serum into dogs. In three out of five experiments there resulted a fall in blood pressure, in the remaining two there was no effect. Gheorghian and Niculescu,[6] working also with serum, found that the blood pressure of the rabbit was uniformly raised as much as 28 mm. Hg by intravenous injection of doses up to 5 c.c., while the injection of similar amounts of serum from persons without hypertension did not influence the pressure. The hypertensive serum was furthermore more toxic for the rabbit than normal serum. Working with vagotomized cats "desensitized" by repeated small intravenous injections of whole blood, Danzer, Brody, and Miles[7] produced a pronounced rise in blood pressure which they attributed to the presence of a pressor substance in the blood of persons with hypertension. Unless the preliminary procedures of desensitization and severance of the vagi were carried out, depressor and bradycardic effects which they considered as due to anaphylactic phenomena, entirely overshadowed the otherwise obvious pressor response. Curtis, Monerieff, and Wright,[8] using a similar technic, were unable to confirm these results. Injections of from 10 to 20 c.c. of whole blood obtained from persons with both normal and elevated blood pressures failed to produce in desensitized cats any pressor effect other than could be explained by the volume of fluid injected.

It was felt that the conflicting results of these investigators justified a repetition of these experiments. An attempt was made to allow for uncontrolled factors by making a comparatively large number of observations and comparing the results with those in a control series. Particular attention was paid to using fresh whole blood for injection, thus avoiding the formation of a vasoconstrictor substance due to clotting. This was a factor not sufficiently considered in many of the experiments described above.

METHOD

The carotid blood pressure of a full-grown rabbit under sodium amytal anesthesia (0.08 gm. per kg. by ear vein) was registered directly with a mercury manometer. The blood to be tested was drawn with a large needle from the anticubital vein and the syringe gently emptied into a flask containing enough sodium citrate to make a total concentration of 0.4 per cent. The blood was brought immediately to the laboratory and injected into the external jugu-

lar vein of the rabbit. The lapse of time from the withdrawal of the blood to its injection was usually about five minutes and never over fifteen minutes. A fresh animal was used for each determination. The amount of blood injected was 3 c.c. per kilogram. The lethal dose of the blood both from persons with normal blood pressure and those with hypertension was approximately the same, namely, 6 to 8 c.c. per kilogram. In the earlier experiments desensitization was attempted by giving repeated small intravenous injections (0.1 to 0.5 c.c.) before the massive dose. In some instances the animals were atropinized and the vagi were severed. These procedures did not appreciably influence the pressor response.

The patients from whom the blood was obtained were carefully studied as to renal function, degree of arterial disease, and height and lability of the blood pressure. With the exception of one patient who had malignant hypertension and renal failure, they belonged to the essential hypertension group with constantly elevated quite stable blood pressures. One patient had a coronary thrombosis resulting in a lasting fall in blood pressure. Similar curves were ob-

TABLE I

ESSENTIAL DATA IN EACH OF 20 DETERMINATIONS USING THE BLOOD OF PERSONS WITH CONSISTENTLY NORMAL OR ELEVATED BLOOD PRESSURES

NORMAL BLOOD PRESSURE	WEIGHT OF RABBIT (KILOGRAMS)	AMOUNT OF BLOOD INJECTED	DESENSITIZED	ATROPINIZED	VAGOTOMIZED	MAXIMUM RISE IN MM. HG	DURATION OF RISE IN SECONDS
		c.c.					
A. L.	3.2	9.6	+	+	+	0	–
K. F.	3.3	9.9	+	+	+	0	–
A. E.	3.2	9.6	+	0	0	0	–
S. W.	2.7	8.1	+	0	0	10.0	25
L. M.	3.6	10.8	+	0	0	6.0	90
F. B.	4.3	12.9	+	0	0	0	–
E. H.	4.1	12.3	+	0	0	4.0	3
A. F.	3.7	11.1	0	+	0	8.0	45
J. B.	3.2	9.6	0	+	0	10.0	90
T. A.	4.5	13.5	0	0	0	10.0	40
						Av. 4.8 ± 1.4	Av. 29.3 ± 9.5
Essential hypertension							
H. T.	4.4	17.6	+	0	0	4.0	30
F. M.	4.0	12.0	0	+	0	8.0	35
E. F.	3.4	10.2	0	+	0	8.0	60
A. S.	3.8	11.4	0	+	0	8.0	60
H. P.	3.6	10.8	0	+	0	16.0	20 min.
L. P.	3.4	10.2	0	+	0	12.0	40
O. D.	4.2	12.6	0	+	0	0	–
T. L.	3.9	11.7	0	+	0	12.0	50
A. H.	3.1	9.3	0	0	0	6.0	40
J. H.	4.0	12.0	0	0	0	6.0	15
						Av. 9.2 ± 1.7	Av. 153.0 ± 110
Malignant hypertension, renal failure							
J. R.	3.6	10.8	+	0	0	12.0	60

tained with his blood both before and after this occurrence. Blood from laboratory workers and medical students with normal blood pressures was used for the control determinations.

RESULTS

In general, immediately following the injection there occurred a moderate rise in pressure of relatively short duration. This was succeeded by a pronounced fall lasting usually some minutes with eventual return to the preinjection level. The data in brief, including the height and duration of the initial rise, are given in Table I. In four of the control determinations no rise was recorded, while every rabbit in the hypertension series responded by an increase in pressure. This increase tended to persist longer in the rabbits receiving such blood than in those receiving blood from persons with normal pressures. The average rise of pressure in the control group was 4.8 ± 1.4 mm. of Hg that of the

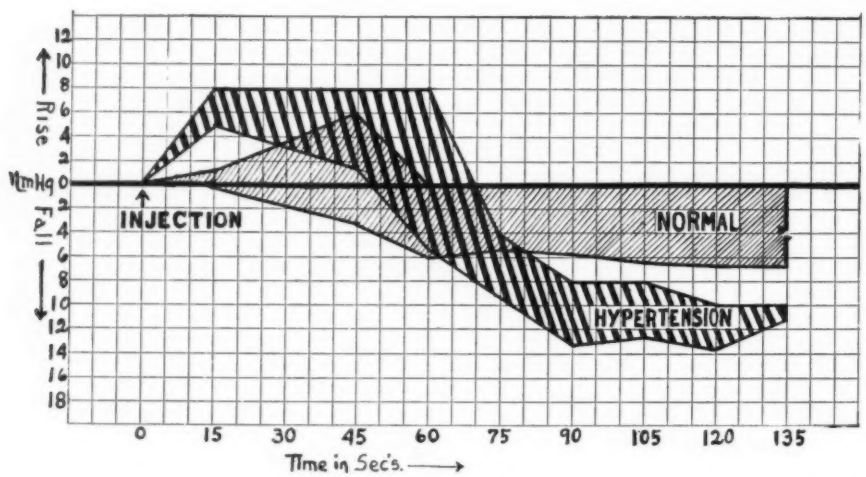


Chart 1.—Blood pressure response of rabbits to the intravenous injection of whole blood from persons with normal and elevated blood pressures.

hypertension group 9.2 ± 1.7, giving a difference in average values of approximately twice the standard deviation of the mean.

The scatter and overlapping of the individual observations are well shown in Chart 1, in which, for each time interval of fifteen seconds, the values lying between the average and the mode are plotted. The chart also shows the secondary fall in pressure which was more pronounced in the hypertension group.

In another limited series of experiments, the effect upon the rabbit's blood pressure of alcohol,* ether, and water soluble constituents of blood from persons with normal and elevated pressures was determined. The detailed data will not

*Bohn, working in Volhard's clinic (see Volhard, F., and Suter, F.: "Nieren und Ableitende Harnwege," 1: p. 422, Julius Springer, 1931), has uniformly produced a pronounced rise in the blood pressure of cats by the intravenous injection of alcoholic extracts of blood from patients with the "pale" type of hypertension as typified by chronic glomerulonephritis and malignant nephrosclerosis. This finding has led Volhard to the conclusion that the elevation of pressure in such patients is the result of vascular spasm due to the presence of a "pressor" or "sensitizing" substance in the circulating blood. Its exact nature remains obscure. The elevation of pressure in essential or "red" hypertension, on the other hand, depends upon a different mechanism as alcoholic extraction of the blood from such patients failed to yield a pressor principle. Our results with this procedure are in conformity with this latter finding, and the patients tested belonged to the essential hypertension group.

be given because the results were uninformative. These fractions caused uniformly a transient depressor effect of approximately the same order of magnitude in all observations.

DISCUSSION

The nonphysiologic nature of experiments of this type, with the unavoidable introduction of uncontrollable factors of obscure nature, was recognized at the outset. It was felt that negative results would not necessarily indicate the absence of a pressor principle in the blood of persons with hypertension, whereas positive results might be significant. The results obtained, while suggestive, are statistically inconclusive because the difference in the average rise of pressure in the two series of observations does not exceed three times the standard deviation of the mean.

The secondary prolonged fall in blood pressure following injection probably represents a reaction to foreign protein which could not be obviated entirely by attempted desensitization or other procedures. That this fall was more pronounced in the group receiving the hypertensive blood is probably attributable to the fact that desensitization was attempted only once in this series as against a total of six times in the control series.

It is fair to conclude from this study that the hypothesis of a pressor principle in the blood stream of persons with consistently elevated blood pressures should not be lightly abandoned but should be subjected to further experimental study for confirmation or refutation.

SUMMARY

The immediate blood pressure response of the rabbit to massive intravenous injection of fresh citrated whole blood from persons with normal and consistently elevated blood pressures was studied.

In most instances a transitory rise of pressure followed by a prolonged fall resulted.

The average initial rise of pressure of the animals receiving blood from patients with hypertension was slightly greater and generally more prolonged than that observed in the control series.

Alcohol, water, and ether soluble fractions of such blood gave uniformly a transitory fall in blood pressure.

The results of this study warrant further investigation as to the possibility of a pressor principle in the circulating blood of persons with arterial hypertension.

REFERENCES

1. Fishberg, A. M.: Anatomic Findings in Essential Hypertension, Arch. Int. Med. **35**: 650, 1925.
2. O'Connor, J. M.: Über den Adrenalingehalt des Blutes, Arch. f. exper. Path. u. Therap. **67**: 195, 1911-12.
3. Hülse, W.: Untersuchungen über Gefässverengerende Stöffe im Blute bei Hypertonien, Zentralbl. f. Inn. Med. **43**: 1, 1922.
4. Kahlson, G., and Werz, R.: Über Nachweis und Vorkommen Gefässverengernder Substanzen im Menschlichen Blute, Arch. f. exper. Path. u. pharmakol. **148**: 173, 1930.

5. Stewart, G. N., and Cushing, H. K.: So-Called Biological Tests for Adrenalin in the Blood, With Some Observations on Arterial Hypertonus, J. Exper. Med. **14:** 377, 1911.
6. Gheorghian, I., and Niculescu, G.: Recherches Experimentales sur la Circulation Generale avec le Serum des Malades Hypertoniques, Compt. rend. Soc. de Biol. **90:** 1163, 1924.
7. Danzer, C. S., Brody, J. G., and Miles, A. L.: On the Existence of a Pressor Substance in the Blood of Clinical Cases of Hypertension, Proc. Soc. Exper. Biol. & Med. **23:** 454, 1926.
8. Curtis, F. R., Monerieff, A. A., and Wright, S.: The Supposed Presence of a Pressor Substance in the Blood of Patients With High Blood Pressure, J. Path. & Bact. **30:** 55, 1927.

MONOCYTIC LEUCEMIA*

W. M. Fowler, M.D., Iowa City, Iowa

SINCE the first description of monocytic leucemia by Reschad and Schilling in 1913[1] many similar cases have been reported in the literature, although hematologists vary in their opinions as to the acceptability of many of these cases. Dameshek[2] in 1930 reviewed the literature on the subject and accepted sixteen of the reported cases and added two others. Additional cases have been reported since that time by Lawrence[3] (three), Clough[4] (one), Cooke[5] (one), Farley[6] (one) and Asselstine[7] (two), bringing the total, with the three cases presented here, to twenty-nine.

With the recent popularization of Pappenheim's technic for staining the leucocytes by means of neutral red and Janus green, there has been added, if not a more accurate, at least another reliable method to aid in the differentiation of leucocytic cells into the various series. This has served to arouse interest in monocytic leucemia, and in several of the more recently reported cases, including two of those presented here, the supravital method of staining has been used.

Since some hematologists dispute the existence of this third type of leucemia, it seems best to present as much proof as possible, in the form of carefully studied cases, that such a condition does exist.

Case 1.—W. I., a white male, aged sixty-four years, was admitted to the University Hospital because of weakness, palpitation and moderate shortness of breath of a few weeks' duration. On examination the heart and lungs were found to be normal except for moderate emphysema. The lymph nodes of the inguinal, epitrochlear and axillary regions were slightly enlarged and firm in consistency. Although the edge of the spleen could be felt the splenic dullness was not increased. The liver was not enlarged.

There was a trace of albumin in the urine. The Wassermann reaction was negative. Gastric analysis revealed an achlorhydria. The bleeding time, coagulation time and prothrombin time of the blood were within normal limits, and the resistance of the erythrocytes to hypotonic salt solution was normal. The blood platelets were diminished.

The patient was given two transfusions, following which he left the hospital but returned within a week because of increasing weakness. There had been no change in the physical signs except for a slight increase in the size of the superficial lymph nodes and of the liver. During the next few weeks a generalized lymphadenopathy developed, the nodes being firm,

*From the Department of Internal Medicine, State University of Iowa.
Received for publication, October 17, 1932.

movable but not tender. The spleen and liver remained unchanged. The gums became spongy and necrotic and the face edematous. Blood cultures were sterile. The patient remained afebrile throughout. He became progressively weaker and died two months after his first admission to the hospital.

Necropsy: The spleen weighed 170 gm. and showed a few hemorrhagic areas beneath the capsule but the splenic tissue was rather pale. The bone marrow was red with small grayish areas. The lymph nodes were enlarged and of a grayish color with small hemorrhagic areas.

On microscopic examination there was an infiltration of all organs by cells having a small amount of eosinophilic cytoplasm and a somewhat notched nucleus. In many of these only a very small bit of cytoplasm could be seen. In addition to the above cells there were neutrophiles and other cells of the myelocytic series. There was a diffuse infiltration of the heart, liver, adrenals, and kidneys. The spleen showed an absence of germinal centers and the pulp was filled with the cells described above. The architecture of the lymph nodes was obliterated by the cellular infiltration and the bone marrow was likewise packed with cells of the same type.

TABLE I

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES	MONOCYTES	NEUTROPHILES	QUESTIONABLE MYELOCYTES	BLAST CELLS	LYMPHOCYTES	UNCLASSIFIED
7/23/28	40	2,530,000	41,550	48	42			10	
7/26/28			49,950	15	25	39	8	12	1
7/27/28	38	1,900,000	40,500	16	41	20	6	11	6
7/28/28			46,950	30	27	20		4	19
7/29/28				31	39	18		10	2
7/31/28	43	2,420,000	47,200	20	41	13			26
8/15/28	36	1,760,000	49,800	13	51	14		22	
8/18/28	25	1,990,000	50,900	17	58	9		7	9
8/22/28	36	1,740,000	66,700	13	53	15		15	4
8/27/28	28	2,790,000	111,000	18	17	52		11	2
9/ 1/28			124,000	32	43	23			2
9/ 8/28		1,040,000	91,750						
9/11/28	20	960,000	142,000	32	20	41		6	1
9/13/28	30	790,000	151,500	21	57	18		4	
9/18/28			285,000						
9/19/28		770,000	223,000						

With Wright's stain most of the nuclei of the monocytes were bean- or kidney-shaped and contained a fine reticulum of chromatin without visible nucleoli. The cell borders were very irregular, sometimes frayed out and in others thrown into many folds. Small projections of cytoplasm were common, some of which were almost pedunculated. The cytoplasm contained small reddish granules. Basophilic polymorphonuclear cells were rare and no eosinophiles were found. All of the myelocytes were of the neutrophilic type. An occasional normoblast was seen but these were never numerous.

At the time this patient came to the hospital we were not convinced that monocytic leucemia existed as a separate form and when first examined the diagnosis was thought to be either myelocytic leucemia or malignant lymphoma with extensive bone marrow infiltration. Repeated blood examinations, however, established the diagnosis of monocytic leucemia. The percentage of monocytes in the blood smear (48 per cent) was not as high as is reported in the majority of cases.

If the supravital staining technic had been used in this case it is probable that many of the cells which have been classified as myelocytes on the dry smear would have been found to be of the monocytic series. The absence of basophiles and eosinophiles together with the size of the spleen and the necropsy report are distinctly against the diagnosis of myelocytic leucemia.

CASE 2.—S. A., a white male, aged forty-three years, was admitted to the University Hospital on Jan. 12, 1931. For five months he had noticed weakness, loss of weight, and an afternoon fever. Pustular lesions appeared on the skin but these underwent a definite change in character shortly before admission when they became firm, indurated, reddish areas without pus formation. Five days before admission he first noticed a swelling of the left lower jaw which gradually increased in size and developed an extremely offensive odor. At the same time a small tender swelling developed under the left mandible.

Examination revealed moderate pallor of the skin and mucous membranes. There was a granular, grayish mass with an indurated border and a necrotic center on the lower left gum. Extending from this lesion was an area of induration leading to an enlarged node in the submaxillary region which was tender, movable, and elastic in consistency. There were palpable nodes in the right cervical, epitrochlear, and inguinal regions. The edge of the liver could just be felt below the right costal margin but the spleen was not palpable. There were red indurated areas of varying size on the forehead, lower face, neck, back, and buttocks. The largest of these was about 3 cm. in diameter.

Course: The lesion in the mouth progressed rapidly and involved the greater part of the lower jaw in spite of x-ray therapy. The sub-mandibular region became swollen and very firm from below the left ear to the angle of the jaw on the right. The skin lesions increased in number, especially about the neck. Some of these became pustular while others remained as indurated areas. Similar lesions appeared in the antecubital fossa at the site of venepunctures. Soft and movable lymph nodes about 1 cm. in diameter developed in both axillae and inguinal regions. The spleen gradually enlarged until it extended two finger-breadths below the left costal margin. The temperature remained at about 103° F. with only slight remissions. The patient received two blood transfusions but grew progressively worse and died Jan. 27, 1931.

Laboratory Examination: The urine showed 1-plus albumin and a few casts. The blood Wassermann was negative. Roentgenograms of the lungs and skeletal system were normal. The bleeding time, coagulation time, constrictor test, and fragility of the red blood cells were normal, but the clot retractility was poor. The platelets were slightly diminished.

TABLE II

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES	MONOCYTES	BAND NEUTROPHILES	SEGMENTED NEUTROPHILES	BASOPHILES	MYELOCYTES	BLAST CELLS	LYMPHOCYTES	TURK IRRITATION CELLS	DEGENERATED CELLS	UNCLASSIFIED
1/12/31	42	3,180,000	76,200	75	3		1	3	1	10	2	5	
1/13/31			80,000										
1/18/31			51,200	75	4	3	1	4	2	6		3	2
1/19/31			50,000	72	1	3		5	3	13	1	2	
1/20/31	48	2,600,000	77,000										
1/21/31			106,000	80		5		3		6	2	4	
1/22/31			49,000	75		6		3		9	1	6	
1/23/31	40	1,730,000	107,000	84		1			3	1	1	10	
1/24/31			112,000										
1/25/31	48	2,500,000	118,000	73		5		6	2	7	2	5	

The monocytes as seen on a dry smear with Wright's stain were of two types: The first was a cell with a regular outline averaging 10 to 15 microns in diameter. It had abundant, clear, grayish lavender cytoplasm and a majority contained small, sharply defined reddish granules. A few of the cells had no granules and some had a cloudy or blotchy cytoplasm. The nucleus occupied about half of the cell area and had a definite fine chromatin network which was purplish in color. There were no large blocks of chromatin material and no visible nucleoli. The nuclei were frequently bean- or kidney-shaped with the cytoplasmic

granules grouped in the bend of the nucleus. Occasionally the nuclei were more irregular and in many cells it appeared to be folded on itself, producing irregular dense areas.

The second type of cell was larger in size and was more numerous. On the periphery of these cells were one or more protrusions or buds of the cytoplasm, the smaller of these being clear and without granules, while the larger ones contained reddish granules in varying numbers. A few vacuoles were found in the cytoplasm, but no inclusion of foreign material was seen. There were fewer granules in this latter type, but the cytoplasm was otherwise like that of the smaller cell. The nucleus was irregular and lobulated, and the nuclear folds were present but less evident. The nucleus stained less deeply but the chromatin network was similar to that seen in the first type. No nucleoli were seen.

Oxidase staining of the smear showed that 1.6 per cent of the white blood cells were oxidase positive, while a similar stain on a bone marrow smear showed 6.2 per cent of the cells to be oxidase positive. A suspension of bone marrow from the femur in blood serum showed that 19.1 per cent of the cells were leucocytes, whereas in a suspension from bone marrow obtained from a rib 32.1 per cent of the cells were leucocytes. This is in keeping with the fact that leucemic infiltration or metaplasia of the bone marrow is most marked in those areas which are normally most active in hematopoiesis.

Supravital staining with neutral red showed about 40 per cent of the cells to have a rosette formation of the red granules about the concavity of the nucleus. Many of the other cells had fine granules evenly distributed throughout the cytoplasm and appeared to be monocytes without the rosette. The granules and their arrangement were distinctly different from those found in the myelocytes and lymphocytes.

Necropsy: The liver was enlarged and soft in consistency, weighing 2900 gm. Both the surface and cut section were yellowish brown in color. The spleen weighed 550 gm., was soft, and on cut section showed numerous large hemorrhages and a destruction of the normal architecture. The kidneys were enlarged and soft, weighing 360 and 420 gm. They were mottled gray in color with numerous pinpoint hemorrhages on the surface. This grayish discoloration with hemorrhagic areas was present throughout the kidney parenchyma. The myocardium was pale and flabby and there were intramuscular hemorrhages. The mediastinal and mesenteric lymph nodes were enlarged and rather firm. The bone marrow of both the femur and ribs was hyperplastic and grayish in color.

Microscopic Examination.—The capillaries of the lungs were packed with leucocytes. These cells, for the most part, were round or oval mononuclear cells which were two or three times the diameter of the erythrocytes. The nuclei were rich in chromatin and many had irregular indented and lobulated outlines. In another section through a bronchopneumonic area an exudate was present which was not made up of polymorphonuclear cells, but consisted for the most part of the mononuclear cells described above. The heart was diffusely but unevenly infiltrated with the same type of cell.

The spleen was congested and in places there were fairly large hemorrhages. It was densely infiltrated by these large mononuclear cells, some of which had a light-staining nucleus while others contained a dark-staining chromatin material. Giant cells containing six or more nuclei of both the light- and dark-staining types were abundant and a few mitotic figures were seen. Few neutrophiles were present. In the liver the same type of cell was present with the most dense infiltration about the portal canals. The tubules of the kidneys were encroached upon by the cellular infiltration. The normal architecture of the lymph nodes was lost and the sections were made up for the most part of mononuclear cells with an occasional giant cell. Practically all organs examined showed a similar histologic picture. No evidence of proliferation of the fixed reticular tissue was found, but the infiltration by the monocytes was so dense that this proliferation, if present, might have been obscured.

CASE 3.—G. H., a white male, aged forty-four years, was admitted to the University Hospital because of an infection in the thenar eminence of his right hand which had followed a minor injury three weeks prior to admission. He developed a fever and lymphangitis which subsided after the application of hot, moist dressings. At the time of the subsidence of the general symptoms he noticed excessive salivation, and a swelling of the left cheek. Four days

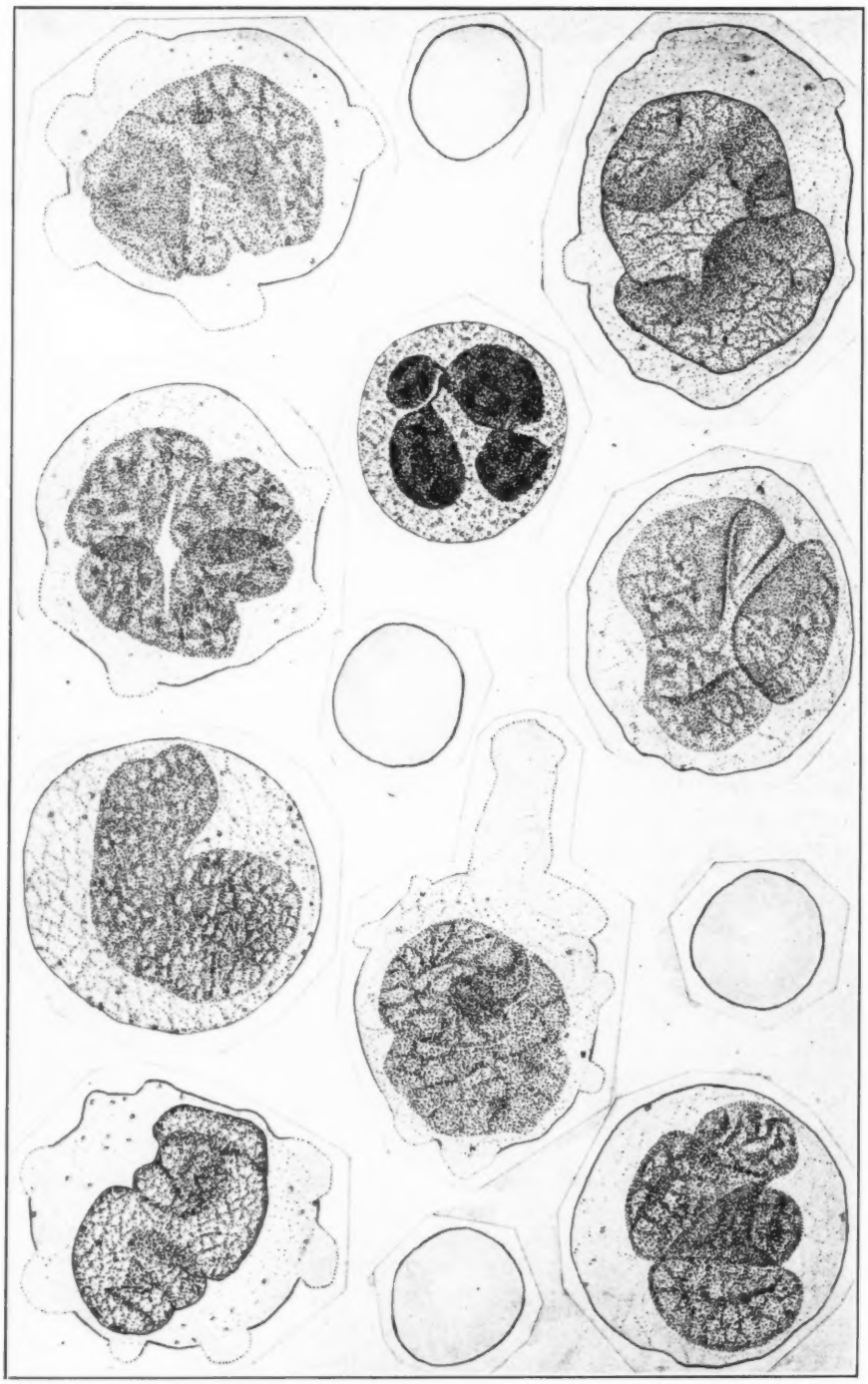


Fig. 1.—Camera lucida drawings of monocytes with erythrocytes and one neutrophile for comparison. Made from a fixed smear with Wright's stain.

later a similar painful swelling appeared on the right side of the face which gradually increased while that on the left disappeared. An abscess on the thenar eminence of the right hand was incised and a small amount of pus liberated.

The patient was extremely weak and at times he was semicomatose or irrational. There was marked pallor of the sclera, skin and mucous membranes but there were no petechiae or other evidences of hemorrhage. There was a swelling of the right cheek extending downward from the malar eminence over the face and submandibular region. The most prominent part of the swelling was just below the level of the line of closure of the teeth. This area was definitely tender to touch and was firm and indurated, but there was no discoloration of the overlying skin. The submandibular nodes were enlarged and firm on both sides but the enlargement was greatest on the right. There was excessive salivation and a very foul odor to the breath. On the inner surface of the right cheek was a grayish brown area of necrosis 1.5 by 3 cm. in diameter. The margins of this area were clear-cut and there was no induration, discoloration or other evidence of infection in the surrounding tissue and it appeared to be purely a necrotic lesion. The inner surface of the cheek was diffusely swollen. The gingivae were swollen, infiltrated, and bled rather easily with pressure, although there were no spontaneous hemorrhages. The gingivae extended upward between the teeth as high as the incisor surfaces in some places.

The posterior cervical, epitrochlear, axillary, and inguinal nodes were enlarged but remained discrete and movable. The cardiorespiratory system was normal. The liver and spleen were not palpable although the area of splenic dullness was definitely increased. At the base of the thenar eminence of the right hand was a red, indurated area with an incision in the center from which a small amount of seropurulent material could be expressed.

Course.—During his first few days in the hospital the patient had a fever which reached 102° F. on several occasions, but later the temperature became subnormal. A blood culture remained sterile. The patient's skin was very warm and moist at all times regardless of his temperature, and he had frequent, drenching sweats. The lesion on the hand improved but those on the gums and cheek remained stationary. He became progressively weaker and the

TABLE III

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES	MONOCYTES	NEUTROPHILES	EOSINOPHILES	BASOPHILES	MYELOCYTES	BLAST CELLS	LYMPHOCYTES	TURK IRRITATION CELLS	DEGENERATED CELLS	UNCLASSIFIED	REMARKS
10/17/31	65	2,800,000	16,000											
10/18/31			29,650	23	55					17		5		
10/20/31		2,500,000	40,000	64	22					11		3		
10/21/31			32,000	74	3				1	17		5		
10/22/31	35	1,690,000	13,850	71	6					22		1		
11/23/31	35	2,230,000	22,450	88	1.5					9	.5	1		
10/24/31	35	2,250,000	25,280	72	3					15		10		
10/25/31			28,350	75	2				2	12		9		
10/26/31			35,200											Transfusion 520 c.c.
10/27/31	35	1,770,000	10,250	56	17		2		3	13		8	1	
10/28/31	40	2,090,000	19,600	65	9			1	4	11		10		
10/29/31			12,200											
10/30/31			11,200											Transfusion 300 c.c.
10/31/31			13,400	54	11	1	1			23		10		
11/ 1/31			13,200	71	7					16		6		
11/ 2/31			11,000	55	11				5	15		14		
11/ 3/31				64	10				1	17		8		

erythrocyte count decreased despite two transfusions. He was mentally depressed and finally demanded his discharge from the hospital. He died at his home on Nov. 23, 1931, about nine weeks after the onset of symptoms.

Laboratory Examination.—Urinalysis showed a trace of albumin but no casts. The blood uric acid was 5.7 mg. per cent, urea nitrogen 14.7, creatinine 1, and cholesterol 59.5. A roentgenogram of the mandible showed no evidence of osteomyelitis. The hematocrit reading was 27 per cent and the blood platelets were normal.

On a fixed smear the predominant cell in this case varied from 10 to 15 microns in diameter, with the nucleus occupying three-fourths or more of the cell area. The configurations of the nuclei were extremely variable; a few were almost circular in outline, but the great majority were very irregular. Even in those with the most regular outline there was usually some degree of indentation, and practically none had a uniform density throughout the nucleus. The nucleus gave the appearance of being folded on itself so that there were sharply outlined dense areas, the outline of which could frequently be followed out to the periphery of the nucleus and found to coincide with a curve of the nuclear outline. Some of the cells had

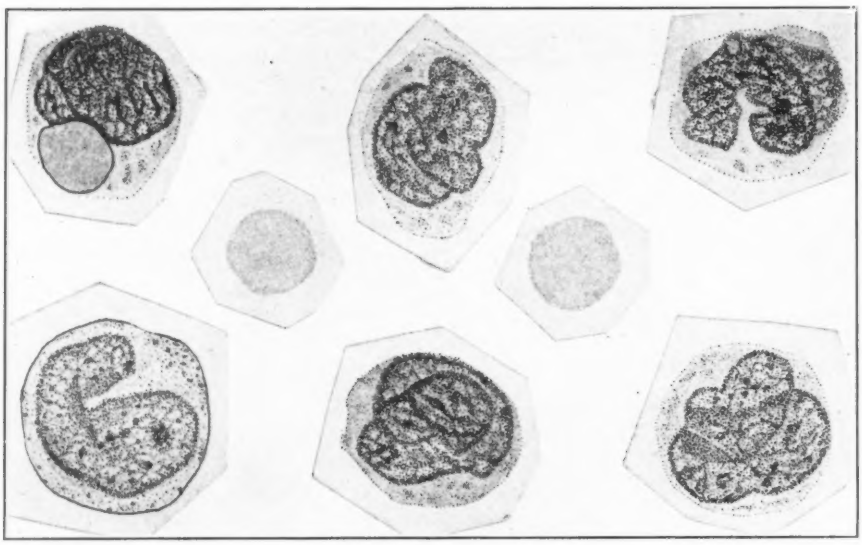


Fig. 2.—Camera lucida drawings of monocytes with erythrocytes for comparison. One monocyte is seen with the inclusion of an erythrocyte. Made from a fixed smear with Wright's stain.

a horseshoe-shaped nucleus which frequently contained nuclear folds. The nuclei in all these cells took a bluish purple tint with Wright's stain. The internal structure consisted of a definite reticular network with rather dense chromatin threads. There was no tendency of the chromatin to form in blocks and nucleoli were extremely rare.

The cytoplasm was basophilic and varied in density from a light hyaline to a dark blue color which was blotchy or coagulated in appearance. There was no perinuclear clear area. In some cells there was only a very narrow rim of cytoplasm, usually visible in two or more places opposite nuclear indentations. A few fusiform cells were present and on a few cells there were small rounded cytoplasmic projections with indistinct outlines which suggested pseudopodia. For the most part the cytoplasm was agranular although a few had small bluish granules. One of these cells was found on a fixed smear with an engulfed red blood cell.

With the vital stains the monocytes were found to have a very indistinct cell outline with many small cytoplasmic protrusions, pseudopodia and elongated processes which were frequently drawn out to a point. The pseudopodia, when present, were at the opposite side of the cell from the nucleus.

The nucleus in most cells was large and irregular in outline and occupied about three-fourths of the cell body.

With neutral red the granules were distinctly different from those of the granulocytic series. The number of granules in the monocytes varied, but for the most part they were numerous and were commonly grouped at one side or in the bend of the nucleus. A few did not have this characteristic rosette, but showed granules scattered throughout the cytoplasm and some cells had a few granules scattered in other areas outside the rosette. The polymorphonuclear granulocytes were extremely active, while motion in the monocytes was sluggish. Large red vacuoles appeared in the cells after a long period of observation.

With Janus green only a few scattered mitochondria were seen. The number, size, and distribution of these did not correspond to those of the lymphocytes, and we were unable to trace any gradation indicating that there were transitional stages between the monocytes and lymphocytes. No cells were found with the very numerous, small mitochondria characteristic of the myelocyte nor were transitional stages between the monocyte and granulocyte noted.[8, 9]

Vacuoles were present in many cells, but none were found with incorporation of other cells or cell fragments, although one such cell was seen on a fixed smear. No amitotic or mitotic figures were seen.

With a combination of Wright's and peroxidose stain on a fixed smear, it was found that 63 per cent of the monocytes contained the peroxidase ferment while 37 per cent had none.

TABLE IV

DIFFERENTIAL COUNT BY MEANS OF NEUTRAL RED

	10/22/31	10/24/31	11/2/31	11/3/31
Monocytes	73	84	55	56
Lymphocytes	22	10	11	12
Granulocytes	5	6	34	32

TABLE V

PEROXIDASE CONTENT OF CELLS

	10/21/31	10/25/31	10/28/31	11/1/31	11/3/31
Much	2	2	11	2	2
Little	16	30	40	40	38
None	82	68	49	58	60

The cells of the last two cases presented several points of dissimilarity and by the existing criteria those in the third case would be classified as the more immature forms of the monocytic series. These cells (Case 3) were somewhat smaller, the cytoplasm was more basophilic and less granular, pseudopodia were less in evidence, and nucleoli, while not common, were more numerous than in the cells of Case 2. We were unable to discern with either Wright's, peroxidase, or supravital stains any convincing evidence of transition between these cells and either lymphocytes or myelocytes.

No conclusions as to the incidence of this condition can be drawn from the number of cases reported in the literature except that it is a relatively rare form of leucemia. There have been admitted to the University Hospital during a fifteen-year period 782 cases of diseases involving primarily the hematopoietic system. Of this group, monocytic leucemia forms only 0.38 per cent, and of the 164 cases of leucemia, it represents 1.82 per cent. There were 28 cases of lymphocytic and 20 cases of myelocytic leucemia to each case of the monocytic form.

The etiology of monocytic leucemia is unknown, and although certain features suggest an infectious basis, as in the case of other forms of leucemia, the neoplastic origin seems most probable.

Certain infectious processes closely simulate the various forms of leucemia and must always be considered in the differential diagnosis. This differentiation is likely to be most difficult in acute infections which produce changes in the blood picture resembling myelogenous leucemia, and in certain of such cases, even the necropsy findings are suggestive of leucemia.[10] Other infectious conditions reproduce a blood picture similar to that of lymphocytic leucemia. The most familiar examples of this are infectious mononucleosis[11] (glandular fever) and whooping cough. In other instances, tuberculosis may produce a leucemoid blood picture of the lymphocytic type. In one case observed personally, a child not only had the blood picture of lymphocytic leucemia, but also the clinical and laboratory findings of hemorrhagic purpura which so commonly occurs in the terminal stages of this form of leucemia. It was only at necropsy that a generalized miliary tuberculosis was demonstrated.

The infectious lesion which most closely resembles monocytic leucemia is Vincent's angina. This condition will frequently produce a mononucleosis with an increased percentage of monocytes or of abnormal mononuclear cells resembling monocytes. The differentiation presents an added difficulty since the organisms of Vincent's angina are almost invariably found on lesions of the mouth regardless of their etiology.

The clinical picture of monocytic leucemia is essentially that of any acute leucemia, as has been emphasized in the literature.[2, 4, 12] Infiltrative lesions of the gums and necrotic areas in the mouth form an especially prominent feature in most cases, and progressive weakness, fever or sore mouth are the most frequent symptoms of onset. On physical examination, enlargement of the lymph nodes may be slight or absent, but is never excessive; the spleen may or may not be enlarged to palpation and percussion, but at autopsy it is usually found to be moderately enlarged. Although an aleucemic form may exist, the leucocyte count is usually elevated and may reach very high levels. The differential count shows a high percentage of monocytes, many of which are of atypical form because of their immaturity. In practically all cases there are some myelocytes. A progressive anemia is a constant feature and a reduction of platelets with hemorrhagic tendencies is a common terminal event.

The origin of the monocyte is not definitely known and a clear conception of monocytic leucemia awaits a better understanding of this problem. A complete review of the subject will not be attempted here but only some of the conflicting opinions presented.

The theory that monocytes are derived from the myeloid series of cells in the bone marrow was held by Ehrlich[13] and by Naegeli[14] who would classify this type of leucemia as "monocytoid myelogenous leucemia." Piney[15] and Hittmair[16] consider these cells to be of myeloid origin and believe that they can demonstrate definite transitional stages between monocytes and myelocytes. These authors hypothesize that monocytes develop from the reticuloendothelial system, but only through the intermediation of the myeloblast. Clough[17] stated

that the term "monocytic leucemia is applied to certain cases in which, at first, large numbers of monocytes appear in the blood. These cases have run the course of an acute myeloid leucemia, with the appearance later of many myeloblasts in the blood." However, he later reported a case of monocytic leucemia[4] and stated that recent studies yielded evidence that monocytes have an independent source of origin from the reticuloendothelial system.

Another view concerning the origin of monocytes is that they develop from the lymphocytic series of cells. Bloom[18] states that monocytes and lymphocytes cannot be differentiated by either the dry, supravital or oxidase stains. He believes that the monocytes develop from lymphocytes within the blood vessels where the flow is sluggish, and that he has traced transitional stages between the two forms. This view is also held by Pappenheim, Ferrata, Maximow, and Arneth.[18]

The belief that monocytes are of independent origin, has ardent supporters who adduce experimental evidence to support their theory. Schilling[19] could find no histologic or hematologic transition between monocytic and myelogenous leucemia and concluded that monocytes had an independent origin. Kellum and Forkner[20] present experimental data to show that the monocytes behave independently of the other series and do not proliferate with either the myeloid or lymphoid cells. Sabin,[21, 22] with her coworkers and others who are working with the supravital stains, claim to be able to identify the monocyte by this means and assign to them an independent origin.

Tissue cultures have yielded nothing to support either theory,[18] since monocytes revert to macrophages in this procedure as do lymphocytes and other specialized cells under the same conditions.

From the evidence at hand the theory of the independent origin of the monocyte seems most tenable, although it has not been definitely proved. This impression must be subject to revision if conclusive proof is forthcoming to show that they originate from a common stem cell through the intermediation of either the myelocytic or lymphocytic series. While it offers no direct proof of their derivation as a separate cell type, the existence of a leucemia with a predominance of monocytes and without marked involvement of, or a transition to, either the myelocytic or lymphocytic series is of some significance.

SUMMARY

Three cases of monocytic leucemia are presented, two of which were studied by means of the supravital stains. The etiology, clinical course and diagnosis are reviewed briefly. The principal theories as to the origin of the monocyte are considered. In the light of our present knowledge, together with the fact that in certain cases of leucemia the monocytes proliferate independently of the other series, we believe it is justifiable to classify them as a separate series of leucocytic cells.

Since this manuscript was prepared additional cases have been reported by Farrar, G. E. and Cameron, J. D.: Am. J. M. Sc. **184**: 763, 1932 and Sydenstricker, V. P. and Phinizy, T. B.: Am. J. M. Sc. **184**: 770, 1932.

REFERENCES

1. Reschad, H., and Schilling-Torgan V.: Ueber eine neue leukamie deuch echte uebergangsformen und ihre bedeutung für die Selbstständigkeit dieser Zellen, München med. Wehnschr. **60:** 1981, 1913.
2. Dameshek, W.: Acute Monocytic (Histiocytic) Leukemia, Arch. Int. Med. **46:** 718, 1930.
3. Lawrence, J. S., Josey, A. I., and Young, M. W.: Monocytic Leukemia, Folia Haemat. **44:** 332, 1931.
4. Clough, P. W.: Monocytic Leukemia, Trans. Assoc. Am. Phys. **46:** 258, 1931.
5. Cooke, W. E.: Acute Monocytic (Histiocytic) Leukemia, Lancet **221:** 129, 1931.
6. Farley, D. L.: Monocytic Leukemia, Med. Cl. No. Amer. **13:** 991, 1930.
7. Asselstine, S. M.: Acute Monocytic and Lymphatic Leukemia, Canad. M. A. J. **26:** 174, 1932.
8. Sabin, F. R., Austrian, C. R., Cunningham, R. S., and Doan, C. A.: Studies on the Maturation of Myeloblasts Into Myelocytes and on Amitotic Cell Division in the Peripheral Blood in Subacute Myeloblastic Leukemia, J. Exper. Med. **40:** 845, 1924.
9. Simpson, M. E., and Deming, J. M.: The Identification of Myeloblasts With Vital Stains, Folia Haemat. **34:** 103, 1927.
10. Krumbhaar, E. B.: Leukemoid Blood Pictures in Various Clinical Conditions, Am. J. M. Sc. **172:** 519, 1926.
11. Baldridge, C. W., Rohner, F. J., and Hansmann, G. H.: Glandular Fever (Infectious Mononucleosis), Arch. Int. Med. **38:** 413, 1926.
12. Merklen, P., and Wolf, M.: Leucémies à Monocytes, Rev. de méd., Paris **45:** 153, 1928.
13. Ehrlich, P., and Lazarus, A.: Die anaemie, Vienna, Alfred Holder, 1909.
14. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, ed. 4, Berlin, Julius Springer, 1923.
15. Piney, A.: Recent Advances in Haematology, Philadelphia, 1931, P. Blakiston's Son & Co.
16. Hittmair, A.: Akute myelose mit monozytoiden zellformen, Folia Haemat. **42:** 271, 1930.
17. Clough, P. W.: Diseases of the Blood, New York, 1929, Harper and Brothers.
18. Bloom, W.: The Origin and Nature of the Monocyte, Folia Haemat. **37:** 1, 1928.
19. Schilling, V.: The Blood Picture, ed. 8, St. Louis, 1929, The C. V. Mosby Co., pp. 212.
20. Kellum, W. E., and Forkner, C. E.: The Monocytes in Experimental Leucocytosis, Folia Haemat. **38:** 198, 1929.
21. Sabin, F. R.: Studies of Living Human Blood Cells, Bull. Johns Hopkins Hosp. **34:** 277, 1923.
22. Sabin, F. R.: Studies on Blood, Bull. Johns Hopkins Hosp. **32:** 314, 1921.

LABORATORY METHODS

A SIMPLE ONE-STAGE TECHNIC FOR HEPATECTOMY IN THE DOG*

With Some Remarks on the Clinical Symptomatology of Terminal Hepatic Insufficiency

J. Markowitz, M.D., Ph.D., M.S. in Exper. Surg., Wallace M. Yater, A.B., M.D., M.S. in Med., and W. H. Burrows, Washington, D. C.

TWO methods are commonly used to excise the liver of laboratory animals. The classical method of hepatectomy devised by Mann (1921) of The Mayo Clinic has proved to be an eminently satisfactory procedure in the hands of those who have taken the trouble to learn it. This method is applicable only to dogs and consists of three separate operations, of which the first two are for the purpose of forcing a collateral circulation to develop for the portal vein and vena cava so that at a final operation the liver may be removed without disturbing the animal in any other way. The first stage of this method consists in a reverse Eck-fistula. Markowitz and Soskin (1927) modified Mann's principle for removing the liver by partially tying the portal vein and vena cava, so that the increased venous pressure caused a similar collateral circulation to develop. At a subsequent operation the liver could be removed, there being sufficient collateral circulation to permit an adequate venous return from the posterior half of the body. Mann and Graham (1928) have made some slight objections to this method on the grounds that it is not always certain that sufficient collateral vessels have developed at the time of the hepatectomy. Markowitz and Soskin were well aware of this but preferred to limit their method to a two-stage operation, realizing that it could always be transformed into a three-stage operation, the second of which would consist in completely tying the partially ligated vessels. In actual practice the two-stage method has worked out very well, not only for dogs but for rabbits, monkeys, and guinea pigs. Drury (1929), for instance, employed a modified two-stage method for removing the liver of rabbits with very satisfactory results, reporting liverless rabbits that lived as long as forty hours.

These methods for removing the liver of dogs entail one or more preliminary operations. It is no injustice to these methods to state that they are very troublesome and for the average physiologic laboratory are not applicable, since they entail the preparation and maintenance of a supply of animals

*From the Departments of Physiology and of Medicine, Georgetown University School of Medicine.

Received for publication, November 27, 1932.

for long periods of time when a research is being done requiring liverless dogs. We thought it would be of value to physiologic workers if a simple one-stage method were available that would permit complete removal of the liver without disturbing the animal in any other way. It is true various procedures are described in the literature for tying off the vessels to the liver after shunting the portal blood to the vena cava, but these methods leave a gangrenous organ in the abdominal cavity of which the toxicity, as Mann and his pupils, and Ellis and Dragstedt (1930), and others have shown, is enormous. In our opinion such experiments are rarely justifiable even apart from the fact that a certain amount of blood reaches the liver through the diaphragmatic veins and through the vena cava during the excursions of the diaphragm.

Firor and Stinson (1929) recently described a one-stage method for removing the liver of laboratory animals by cannulating the vena cava above the liver, the portal vein and the vena cava below the liver, and after this was completed, removing the liver by stripping from the cannula between these points. For this operation a Y-shaped cannula was used. The dogs lived from twelve to sixteen hours. Firor and Stinson were probably not dealing with animals that died of uncomplicated hepatic insufficiency. The operation of hepatectomy is in itself sufficiently disturbing to the animal without the added burden of complete obstruction to the portal vein and vena cava in the interval that these structures are being connected by a cannula. This method for removing the liver may be recommended to those who have not mastered the technic for doing an Eck-fistula. We believe the method described by us to be a superior one. The operation is easier, and the animals live longer and in better condition.

One of the great difficulties attending the final operation of hepatectomy by the multiple-stage method has been hemorrhage during the final removal of the liver. This is due to the abundance of new anastomotic channels in the peritoneal adhesions. In the hands of the inexperienced this difficulty is generally an insuperable one and accounts for the relatively short survival period reported by most workers who have dehepatized dogs. One of the merits of a one-stage technic for hepatectomy is the absence of hemorrhage from this source.

It is one purpose of this paper to describe a thoroughly tried, simple method for excising the liver of a dog at one operation, which lasts about forty minutes. The operative mortality is slight, once the technic has been mastered. This method is based on the principle described by Firor and Stinson that a glass cannula of wide caliber may be inserted in the inferior vena cava without the development of any clotting whatsoever over a period of twenty-four hours. In the dog some hours following hepatectomy the clotting power of the blood becomes diminished, partly due to its lowered fibrinogen content. Consequently one may predict that the inferior vena cava traversing the liver may be reconstructed by a glass tube without signs of clotting in the tube. At first view this observation appears irreconcilable with the current knowledge of the clotting of blood. Perhaps it may be explained on the grounds that normal blood contains traces of heparin and antithrombin which, when

the minute volume of blood flow is great enough, will suffice to prevent coagulation over a smooth foreign surface, such as a glass cannula. The principle for our method of hepatectomy, as illustrated in Fig. 1, is to tie a paraffined Pyrex glass cannula into the inferior vena cava, inserting it and tying it just below the liver. The glass cannula projects into the thoracic portion of the inferior vena cava. Blood from the hepatic veins can flow uninterruptedly around the paraffined glass surface. An Eck-fistula is then rapidly performed so that the portal stream is completely diverted to the vena cava below the cannula. The structures in the lesser omentum are next tied and the vena cava between the liver and the diaphragm is securely ligated over the cannula. The liver is then removed by several cautious cuts of the scalpel.

It might be suggested that the removal of the liver in one stage is too drastic a procedure. The subsequent behavior of such dehepatized dogs, however, compares favorably with the best prepared by the multiple-stage method. As a matter of fact, the preparations are usually in much better condition than the average animal dehepatized by the older methods.

We are proposing this method as a preferable one to the multiple-stage methods for 90 per cent of experiments requiring the use of liverless dogs. When the investigation concerns the coagulation of blood, it would be wise to accept with reserve conclusions drawn from animals dehepatized according to this method. Such experiments should be confirmed on preparations from which the liver has been removed after forcing the development of a collateral circulation for the portal vein and inferior vena cava. This is preferable, probably, to endothelializing the glass cannula by threading a strip of vena cava through it and reflecting it back over the ends.

TECHNIC OF THE OPERATION

The animal should be fasting for at least twenty-four hours. This is important if a long-lived preparation is wanted.

The first step in the operation consists in aseptically exposing the inferior vena cava just below the liver, ether anesthesia being employed. A midline incision is preferable, since it saves time and avoids some loss of blood from the incision which an S-shaped incision always entails. After the insertion of a wide, self-retaining retractor, the intestines are collected in the hollow of the duodenal mesentery. They are then wrapped in hot towels and packed off from the operative site as described by Fishback (1927) in his account of The Mayo Foundation technic for the establishment of an Eck-fistula. The hepatorenal ligament is cut. The inferior vena cava is freed from its attachments to the posterior body wall between the lumboadrenal vein and the point where the vena cava becomes buried in the liver. Three ligatures are passed under the inferior vena cava, the middle one consisting of strong packing cord about 2 mm. in diameter. The caudad ligature is a temporary one to prevent escape of blood during the cannulation. It is tied tight over a small brass rod as near the lumboadrenal vein as possible. With the left index finger under the vena cava and occluding it, a small cut is made with scissors in this vessel about 5 mm. below the liver. A paraffinized Pyrex glass cannula about 8 cm. long and of suitable diameter is inserted into the vena cava through this opening, air embolism being prevented by the operator holding his thumb over the protruding end. The assistant meanwhile raises the flap of the vena cava by a mosquito forceps. The cannula is pushed into the vena cava as far as it will go, and the caudad end is then made to engage in the freed segment of the cava by pulling gently on a thread tied around the cannula about 1.0 cm. from its lower end (see Fig. 1). As soon as it engages, the previously placed ligatures are tied by the assistant on each side

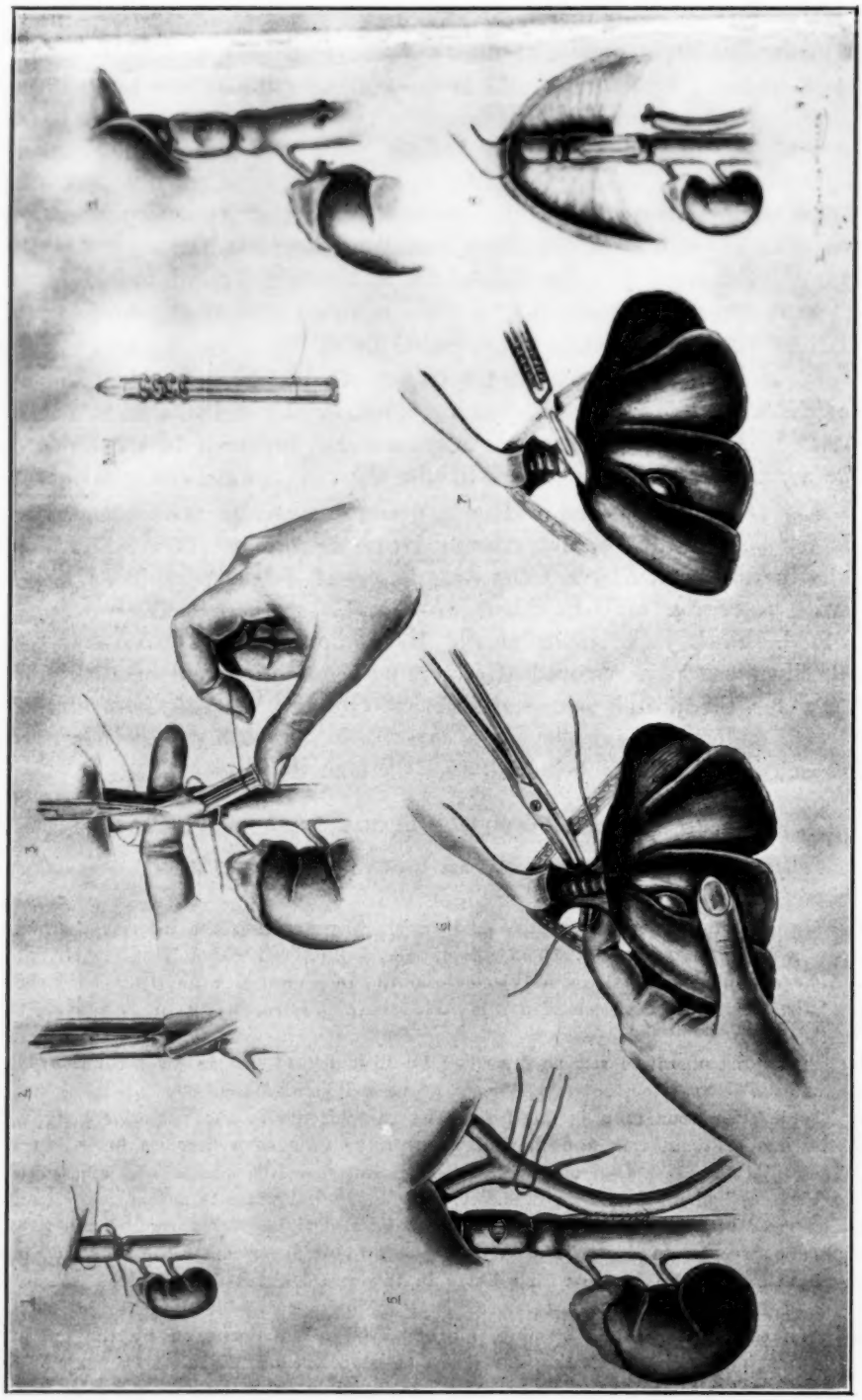


Fig. 1.—Steps in One-Stage Technic for Hepatectomy in the Dog. (Diagrammatic) *1, 2, 3,* and *4* illustrate technic of isolating vena cava and inserting cannula (*3a*). *5* shows Eck-fistula completed. *6* and *7* show placing of ligatures on diaphragmatic cava. *8* illustrates general appearance of diaphragmatic area after removal of the liver.

of the opening in the vessel. The ligature over the brass rod is now quickly cut, thus relieving the obstruction to the inferior vena cava. The complete obstruction of the vena cava need not last more than ninety seconds.

The second step of the operation consists in clearing away the tissue over the portal vein and establishing an Eck-fistula. This is performed by the ingenious method of Fischler and Schröder (1909), as described by Fishback. The Eck-fistula should be rather low (at least 1 cm. from the edge of the cannula) and the stoma should be as large as possible. To make an Eck-fistula in a seven kilo dog is quite practicable, but animals smaller than this are not recommended. For a seven kilo dog the stoma should be about 2 cm. long. It is possible to complete the operation successfully with a smaller stoma, but we have always felt that in such cases there may be a certain amount of back pressure in the portal vein. In placing the suture lines it is advantageous not to sew into the tributaries of the portal vein at the caudad extremity of the stoma.

The third part of the operation consists of the hepatectomy proper. The portal vein is doubly tied at a safe distance above its last tributary (the pancreaticoduodenal vein) and cut between the ligatures and a Carmalt clamp. The three peritoneal folds binding the liver to the diaphragm are exposed and carefully cut. A packing cord ligature (2 mm. in diameter) is now placed around the vena cava between the diaphragm and the liver. This maneuver should be accomplished without exerting traction on the Eck-fistula, by introducing the left hand into the right subdiaphragmatic region. The ligature follows the hand as it is withdrawn from this track. The operator pulls the liver gently in a caudad direction as his assistant ties the ligature as tightly as possible around the cannula. The ligature must be tied below the white margin of the diaphragm's junction with the cava. Three or four more ligatures are now tied to reinforce this one. The first loop of each tie is held taut by a mosquito forceps as the tie is completed. It is important in placing these ligatures to bear in mind the existence of the small lobe which is tucked away dorsally along the vena cava close to the esophagus.

The liver is now removed with several cautious sweeps of the knife. This removes the strip of vena cava over the cannula which is left bare. The last trace of liver tissue which sometimes adheres to one of the ligatures must be carefully removed.

There is often some bleeding from the diaphragmatic veins which have been torn as they course through the coronary ligaments. They should be picked up and tied. Bleeding does not occur from the Eck-fistula, either at the time of the operation, or at any time thereafter.

We strongly recommend at this stage that one or two toy balloons be placed in the hepatic site. The balloons are inflated so that their volume is approximately that of the liver.

The fourth stage consists in sewing up the incision and placing the animal in a warm environment until recovery is complete. This is rapid and usually sthenic in nature. Usually the animals are surprisingly vigorous, walking about the room and displaying the normal curiosity concerning the objects in their environment.

POSTOPERATIVE BEHAVIOR

Such a preparation should live in good condition for about seventeen hours, if the nature of the experiment is such as not to injure the animal's vitality and providing glucose is given intravenously. It may live twenty or more hours, but this is unusual. For the first ten hours or so, hourly injections of one-quarter gram of glucose per kilogram should be given intravenously. Beyond this time the quantity of glucose necessary to maintain the blood sugar at a normal value has to be doubled, and if the animal lives over sixteen hours, it may have to be trebled, since hypoglycemic symptoms ultimately ensue at a higher blood sugar level than the clinical symptoms would suggest.

Ultimately, after a number of severe attacks of nausea and vomiting, the animal develops coma, which may be broken by remissions during which the dog gets up and walks about its cage. Pronounced tachycardia exists. There is an abnormal pinkness of all the visible mucous membranes, as though indicative of widespread vascular relaxation. The temperature is elevated. Soon the breathing becomes very noisy and may be irregular. Usually the pupil is much constricted. The combination of constricted pupils with flushed oral mucous membranes differentiates this condition of terminal hepatic insufficiency from gross internal hemorrhage. The arterial blood pressure at this time, as measured in one dog, was 65 mm. mercury. The limbs show a peculiar rigidity with exaggerated tendon reflexes.

This type of coma may last several hours, or only a few minutes, before the animal gives a convulsive gasp or two and dies. Usually the heart beats for some time after respiration ceases.

At autopsy the fatty tissues of the body are jaundiced. There is always some bloody fluid in the peritoneal cavity which in well-prepared specimens, weighing about 12 to 13 kilograms, is usually about 150 c.c., and which on centrifuging can be demonstrated to be about one-seventh erythrocytes. The visceral peritoneum shows extensive capillary hemorrhages. Usually the mucosa around the orifices of the pancreatic and biliary ducts is hemorrhagic, sometimes extremely so. This finding is more marked when the stoma of the Eck-fistula is undersized, but may be present to a marked degree when it seems as though the stoma is more than ample. The hemorrhagic state of the visceral peritoneum is very characteristic and is more obvious where this membrane has been abraded by indelicate handling during the hepatectomy. The autopsy is otherwise negative except for the occurrence of hemorrhages in various organs, which are especially constant in the subendocardial surface of the ventricles. The glass cannula is free of clot even when some of the shed blood contains enough fibrinogen to clot quickly in a test tube.

If, after the hepatectomy the animal has been gently catheterized, subsequent specimens of urine will contain blood. This is constant even when a soft rubber catheter is used. It is an expression of the hemorrhagic tendency which is so prominent a feature of severe hepatic insufficiency. At autopsy hemorrhagic areas in the bladder mucosa will be noted.

Certain clinical features presented by a liverless dog may now be mentioned. At first the flow of urine is normal. Usually after the eighth hour the quantity of urine obtainable is scanty, even when large amounts of fluid are given intravenously. We have a feeling that this oliguria is partly related to the washing out of urea, of which the formation, as The Mayo Foundation studies have shown, is in abeyance after the hepatectomy. It may, of course, be due to a lowered arterial blood pressure in consequence of a gradual arteriolar relaxation. At any rate, large quantities of urine (50 to 75 c.c. each hour) are obtainable if the animal (in the oliguric stage) is given hourly 100 mg. urea per kilogram and 1 gm. of sodium sulphate per kilogram, both dissolved in about 75 c.c. of water.

One of the striking and constant features about a liverless dog is the tachycardia. Immediately after hepatectomy the pulse rate is high until the

anesthetic has been largely blown off. Ninety minutes after hepatectomy in a 13 kilogram male bull terrier it was 124 (with the animal resting on its side). It hovered around this figure for three hours. Four hours after hepatectomy it was 165, around which figure it hovered for an hour or so. Seven hours after hepatectomy it was around 180. An hour later it was around 200, and by twelve hours after removal of the liver it was 280 per minute. Fourteen hours after hepatectomy it was around 260 per minute. An hour later it was 300 per minute. Ten minutes before death (twenty hours after hepatectomy), the heart rate was 260 per minute.

These facts indicate that the tachycardia, being of gradual onset, is not the immediate result of the intraabdominal surgery. There is reason to believe that it is not due to an oversecretion of adrenalin, for such quantities of adrenalin would cause dilatation of the pupil and pallor of the visible mucous membranes. Moreover, these facts were manifested by an adrenalectomized liverless dog.

Roughly speaking, the intensity of the tachycardia is proportional to the severity of the animal's general condition. It is conceivable that it is reflex, dependent simply on a low blood pressure. The tachycardia of simple hypotension, however, is rarely as marked as that of hepatic insufficiency. Also, we have positive evidence that the rapid pulse rate is independent of the nervous system, and is a direct result of metabolic changes ensuing in the liverless animal. This feature will be discussed in a future paper. We are here drawing attention to its great constancy, and to its approximate value as a criterion of the extent of the hepatic insufficiency.

Vomiting is another characteristic symptom of severe hepatic insufficiency in the liverless dog. Sometimes it is severe and intractable, the animal standing in the corner of its cage and retching steadily for several minutes about four times an hour. In our experience, vomiting always occurs if the animal lives long enough, and severe retching may occur intermittently for five hours. The act may exhaust the dog. Thus one of our dogs retched violently, and was dead five minutes later. We suspected in this instance that a diaphragmatic ligature had slipped, allowing bleeding from the cut vena cava, but the autopsy was negative.

The other symptoms of severe hepatic insufficiency, such as jaundice, hypoglycemia, etc., have been adequately commented on by Mann and Magath. The terminal picture of hepatic insufficiency presents the following characteristic symptoms: Tachycardia (with hypotension); jaundice; severe vomiting; hemorrhagic diathesis; oliguria; and (as Mann and others have shown) certain characteristic changes in the composition of the blood, indicative of the rôle played by the liver in uricolysis, deamination and urea formation, the synthesis of blood fibrinogen and of blood sugar. These changes in aggregate undoubtedly tax the physiologic adjustments of the animal to the utmost, and the question may be asked whether the terminal coma is dependent on the combined trauma to the organism inflicted by these changes or whether "second-stage" hepatic insufficiency (Mann) is due to a specific abnormality, comparable to the hypoglycemia which is undoubtedly the cause of "first-stage" hepatic insufficiency. Firor and Stinson stated that the ultimate cause of

death following hepatectomy is not clear. They ventured that it might be due to progressive heart failure, preceded by vomiting. The question must be left unsettled for the present. The terminal symptoms are certainly not due to absence of glutathione from the blood (as Cahoon has shown in this laboratory).

We have encountered the supposedly characteristic rigidity and noisy, irregular breathing in a comatose dog, five hours after hepatectomy, when the autopsy disclosed that death was due to a slipped diaphragmatic ligature, with severe hemorrhage. McMaster and Drury (1929) failed, many hours after hepatectomy, to benefit liverless rabbits by connecting them to normal rabbits by means of a cross circulation, which is good evidence that the terminal symptomatology is not due to the absence of a chemical from the blood or tissues. They look upon the cause of death as an irreversible change, resulting from a perverted metabolism consequent upon removal of the liver. However, the question of whether the ultimate cause of death following hepatectomy is due to a deficiency of a chemical, or to a surplus of one or more noxious chemicals, must be left undecided for the present.

SUMMARY

A one-stage technic for excising the liver of dogs is described.

The symptomatology of dogs with terminal hepatic insufficiency is described.

At our request, Dr. F. C. Mann kindly investigated the feasibility of our method. He reported that it was feasible and satisfactory in his hands.

REFERENCES

Drury, D. R.: Total Surgical Removal of Liver in Rabbits, J. Exper. Med. **49**: 759, 1929.

Drury, D. R., and McMaster, P. D.: Glucose Requirement of Hepatectomized Rabbits and Its Relation to Lactic Acid Production, Proc. Soc. Exper. Biol. & Med. **27**: 48, 1929.

Ellis, J. C., and Dragstedt, L. R.: Liver Autolysis in Vivo, Arch. Surg. **20**: 8, 1930.

Firor, W. M., and Stinson, E., Jr.: Total Extirpation of Dog's Liver in One Stage, Bull. Johns Hopkins Hosp. **44**: 138, 1929.

Fischler, F., and Schröder, R.: Eine einfachere Ausfuhrung der Eck'schen Fistel, Arch. f. exper. Path. u. Pharmak. **61**: 428, 1909.

Fishback, F. C.: Anastomosis of Portal Vein with Inferior Vena Cava; Thoroughly Tested and Satisfactory Method of Making Eck Fistula, Ann. Surg. **86**: 436, 1927.

Mann, F. C.: Studies in the Physiology of the Liver: 1. Technic and General Effects of Removal, Am. J. M. Sc. **161**: 37, 1921.

Mann, F. C., and Graham, A. S.: Surgery of Liver with Special Reference to Its Removal, Internat. Abstract Surg., pp. 176-182, September, 1928.

Markowitz, J., and Soskin, S.: A Simplified Technique for Hepatectomy, Proc. Soc. Exper. Biol. & Med. **25**: 7, 1927.

THE DETERMINATION OF NONPROTEIN NITROGEN WITH SPECIAL REFERENCE TO THE KOCH-McMEEKIN METHOD*†

CORNELIUS A. DALY, M.S., ALBANY, N. Y.

THE determination of nonprotein nitrogen by the Folin method[1] is, perhaps, the most difficult of the routine analyses made on blood. Inexperienced technicians, especially, have a great deal of trouble in obtaining clear solutions free from particles of silica. When Folin's original instructions are followed, it is almost impossible to avoid the separation of silica from the glass during digestion. This is the result of heating the digestion mixture for too long a period. However, if the acid mixture is heated rapidly until it has a light green color there is much less danger of the glass being attacked.

We have found that the best results are obtained by quickly boiling down the solution with a high flame until the ignition tube is completely filled with dense white fumes of sulphur trioxide. Then the flame is partly reduced so that the digestion mixture still boils briskly. The heating is continued just to the point where the material has a light green appearance. The exact depth of color can only be determined by experience. It is essential to use a good quality quartz pebble to prevent "bumping," since the ordinary grade of glass beads is readily attacked by the phosphoric acid in the digestion mixture.

Although this technic eliminates turbidity due to silica it is open to several objections. In the first place there is the possibility that the oxidation will not be complete in the shorter digestion period. Also, close attention and careful regulation of the flame are essential. For this reason it is difficult for one individual to run more than two or three determinations at a time. Even though adequate care is used, an occasional determination will require repeating or centrifuging because of the action of the digestion mixture on the glass.

If the solution is nesslerized while turbid, there is an increased tendency for the nesslerized solution to become "cloudy" due to a slight precipitation of the reagent. When this occurs, after centrifuging the top of the white layer of silica precipitate will appear red. If this happens the determination must be rejected because the results will be low. On the other hand when the nesslerized solution is read in the colorimeter while turbid, either from a precipitation of the reagent or from silica, the results will be too high. The practice of many technicians in reading nesslerized solutions which are not entirely clear is likely to confuse the physician in "borderline" cases of nitrogen retention.

Because of the disadvantages inherent in the Folin-Wu[1] procedure, many other methods and modifications have been proposed. The procedures of Koch-

*From the Department of Biochemistry, Union University Medical Department, Albany Medical College.

Received for publication, November 10, 1932.

†Research sponsored by The Leopold Schepp Foundation, New York.

McMeekin[2] and Wong[3] seem to be the most practical of these methods. Koch uses hydrogen peroxide for the oxidizing agent, while Wong employs potassium persulphate. Since both of the methods use only sulphuric acid for the digestion mixture, there is no danger of detaching silica from the digestion tubes.

However, when sulphuric acid with peroxide or persulphate is used for the digesting agent, a precipitate of tungstic acid nearly always appears, either during digestion or after water is added. The color of the particles of tungstic acid may be white, gray, yellow, or yellowish green. These compounds, as emphasized by Morley,[4] are very soluble in sodium hydroxide. Therefore the turbidity due to tungstic acid disappears when the solution is nesslerized.

Although the alkaline Nessler's solution readily dissolves the tungstic acid, the reagent itself is affected in the process, so that the nesslerized solution is usually somewhat turbid. Therefore the methods of Koch and Wong have a very limited value in the determination of nonprotein nitrogen when the filtrate contains significant amounts of sodium tungstate. However, with trichloracetic acid filtrates the above methods give excellent results.

As a matter of fact when only nonprotein nitrogen determinations are to be made trichloracetic acid is preferable to tungstic acid for removing the proteins. The solution filters faster and a larger yield of filtrate is obtained. Hiller and Van Slyke[5] have shown that a 2.5 per cent trichloracetic acid precipitating mixture gives the same values for nonprotein nitrogen as tungstic acid.

We studied the problems involving tungstic acid precipitation and silica formation by using the Folin[1] tungstate precipitating solution with a standard nitrogen solution instead of blood. In this way the maximum effect of the tungstic acid was produced since the precipitating blood proteins remove varying amounts of the tungstic acid. We found that sulphuric acid had little power to hold tungstic acid in solution. On the other hand phosphoric acid proved very effective in preventing the precipitation of tungstic acid.

It was thought that the advantages of both the Koch and Folin methods could be combined by using peroxide for the oxidizing agent and just enough phosphoric acid to keep the tungstic acid in solution, yet not sufficient to attack the glass. This combination did not prove entirely satisfactory because the new digestion mixture often removed extremely fine particles from the ignition tube, even though small amounts of phosphoric acid were present.

Folin[6] recently reported that gum ghatti was very efficient in stabilizing colloidal solutions. Later Looney[7] and Folin[8] found that the gum was a very satisfactory protective agent in the direct nesslerization of nitrogen solutions.

We have worked out a modified Koch method in which gum ghatti and sodium citrate are used to prevent the precipitation of the color reagent when solutions turbid from tungstic acid are nesslerized. Hubbard[9] uses Rochelle salt to prevent the precipitation of Nessler's solution but we find that sodium citrate is somewhat more efficient in our modified method. However, neither salt has very much protective power when used alone. As a matter of fact, nesslerized standard solutions of nitrogen do not seem to be quite as clear when either of the salts is present. Nevertheless, we believe that sodium citrate and gum ghatti together give better results than when the gum is used alone. In

order to simplify the procedure when many determinations are being made, the digestion mixture is made up with hydrogen peroxide. This peroxide-sulphuric acid solution will remain serviceable for at least a week at room temperature under ordinary laboratory conditions.

The instability of the digestion mixture is not a serious disadvantage since a weak solution can be readily made stronger by adding a little more peroxide and sulphuric acid. However, if only an occasional determination is made, it is not worth while to make up the digestion mixture containing the oxidizing agent. The acid mixture No. 1 is used and the material oxidized during digestion, without removing from the flame, by adding a drop of 30 per cent hydrogen peroxide as soon as the contents of the tube become brown, just before the white fumes appear.

We have found, as have others, that Koch's Nessler's solution is more satisfactory than Folin's. Koch[2] used 12 c.c. of his reagent for a determination when the digestion mixture contained 50 per cent concentrated sulphuric acid. However, we prefer to use a digestion mixture containing 45 per cent sulphuric acid and to dilute the Nessler's solution so that 15 c.c. will give the same final alkalinity as 12 c.c. of the original reagent. This dilute Nessler's solution can also be used in the regular Folin method.

In the Folin procedure the Nessler's solution is usually measured in a cylinder and quickly added to the 35 c.c. of nitrogen solution in the ignition tube. Then the tube is stoppered and the solution is mixed. The results are usually satisfactory when this procedure is carried out quickly. The density of the Nessler's solution is enough to cause, when quickly added, sufficient mixing to prevent a precipitation of the reagent. We prefer to nesslerize by pouring the reagent, and the sample diluted to 35 c.c., simultaneously into a large beaker and then pouring back into the tube. In this way the two solutions are uniformly mixed and there is less danger of turbidity. Also it saves the extra step of stoppering and inverting the tubes in order to mix. Ordinarily the same beaker may be used for all of the nesslerizations without introducing significant error. It is necessary to employ this technic when using the dilute Koch reagent since its density is insufficient to obtain satisfactory results when the nesslerization is made directly in the tube.

REAGENTS

A stock solution of sulphuric acid is prepared by adding 225 c.c. of C.P. concentrated sulphuric acid to 245 c.c. of distilled water in a liter flask. The acid should be added a little at a time and the solution cooled by rotating the flask in a stream of cold water. From this stock solution of sulphuric acid, two acid mixtures are prepared.

Digestion mixture No. 1 is made up by adding 6 c.c. of water to 94 c.c. of the stock sulphuric acid solution. This acid mixture does not contain peroxide and is used for making up the standard, and also when the oxidizing agent is added separately to the material being oxidized.

Digestion mixture No. 2 is prepared by adding 3 c.c. of 30 per cent hydrogen peroxide to 47 c.c. of the stock sulphuric acid solution. If more convenient, 3 per cent peroxide may be used. In this case 45 c.c. of ice cold C.P.

concentrated sulphuric acid is slowly added to 55 c.c. of ice cold 3 per cent hydrogen peroxide. Acid mixture No. 2, containing peroxide, is used for digesting the sample.

Before using, it is advisable to titrate the two acid mixtures (peroxide does not interfere with the titration) with the modified Koch Nessler's solution. One cubic centimeter of the mixture requires from 10.1 to 10.7 c.c. of the Nessler's reagent, phenolphthalein being used as an indicator. Also, the amount of peroxide in digestion mixture No. 2 can easily be checked. One cubic centimeter of the peroxide mixture should decolorize about 9.5 c.c. of 0.1 N potassium permanganate. When the digestion mixture decreases in strength until less than 4.5 c.c. of potassium permanganate is used up, more peroxide and sulphuric acid must be added in the proportion, 1 c.c. of 30 per cent hydrogen peroxide and 0.8 c.c. of concentrated sulphuric acid to 25 c.c. of weak digestion mixture.

For the standard nitrogen solution weigh out 0.236 gm. of ammonium sulphate or 0.1911 gm. of ammonium chloride, dissolve in a little water and dilute to 1000 c.c. Three cubic centimeters of this solution are equivalent to 0.15 mg. of nitrogen.

The 1 per cent solution of gum ghatti is made by selecting 1 gm. of the gum free from dirt and suspending it in a piece of gauze at the top of 100 c.c. of water in a cylinder. After standing for ten or twelve hours the bulky residue in the bag is discarded and the clear gum solution used as it is. Best results are obtained when the gum is kept at room temperature without preservatives and made up fresh each week.

For the modified Koch Nessler's solution, dissolve 22.5 gm. of iodine in a liter Florence flask containing 20 c.c. of water and 30 gm. of potassium iodide. When the material has dissolved, remove 0.5 c.c. of the iodine solution and save for adjusting the reagent later in the procedure. Now add 30 gm. of pure metallic mercury to the solution in the flask and rotate the contents rapidly, keeping the mixture from becoming hot by holding under a stream of cold water from time to time. Continue shaking the flask until the supernatant liquid has lost practically all of the reddish yellow color due to iodine. Allow the particles to settle for a few minutes, then carefully decant the supernatant liquid and test for the presence of iodine by adding a few drops to a 1 per cent starch solution on a spot plate. Unless a positive test is obtained the solution may contain mercurous compounds. If the test is negative, add a few drops at a time of an iodine solution, using the 0.5 c.c. which was set aside, until a very faint test for iodine is obtained. Dilute at once to 200 c.c. and mix. To 975 c.c. of an accurately prepared 10 per cent sodium hydroxide solution add the entire amount of potassium mercuric iodide solution prepared above. Now add 27 c.c. of 10 per cent sodium citrate solution and dilute to 1600 c.c. Mix thoroughly and allow to clear by standing.

If this Nessler's solution is to be used instead of Folin's reagent in the regular Folin method, the sodium citrate is omitted. The alkalinity of the Nessler's solution should be checked by titrating against 20 c.c. of normal hydrochloric acid, using phenolphthalein as indicator. From 12 to 13 c.c. of the reagent will be required to neutralize the acid if the alkalinity is satisfactory.

PROCEDURE

To 5 c.c. of blood filtrate in a Pyrex ignition tube graduated at 35 and 50 c.c., add 1 c.c. of the sulphuric acid-peroxide digestion mixture No. 2 and two glass beads or a quartz pebble. Heat with the full flame of the micro burner until the dense white fumes of sulphur trioxide appear. Close the mouth of the tube with a small funnel and regulate the flame until the digestion mixture barely boils. At this point the material should be completely oxidized and the digestion mixture will be colorless. However, the presence of a tungstic acid precipitate will give a white, yellow or yellow green appearance to the contents of the tube. If there is a definite brown color due to unoxidized material, the digestion mixture is too weak and more peroxide must be added. Continue the very gentle heating for about two minutes, then remove from the flame. There are usually significant amounts of peroxide present at the end of the digestion but they do not interfere when the solution is nesslerized. When occasional determinations are made it is more convenient to use digestion mixture No. 1 and add the oxidizing agent separately. Either 1 drop of 30 per cent or 10 drops of 3 per cent hydrogen peroxide or 15 drops of a saturated solution of potassium persulphate may be used. In either case the oxidizing agent is added during digestion, just when the contents of the tube become brown. It is not necessary to remove the tube from the flame when adding the peroxide in this way since the digestion mixture still contains enough water to prevent spattering. The small funnel is then placed in position and the digestion completed as described above.

When the digested material is cold, rinse the funnel, both inside and out, add 1 c.c. of 1 per cent gum ghatti and dilute to 35 c.c. In another tube place 1 c.c. of the digestion mixture without peroxide, No. 1, 3 c.c. of the standard nitrogen solution and 1 c.c. of gum ghatti and dilute to 35 c.c. Measure out 15 c.c. of the Nessler's solution with sodium citrate in a small cylinder. Now quickly pour the Nessler's reagent and the 35 c.c. of nitrogen solution simultaneously into a 400 c.c. beaker, whirl and pour back into the graduated combustion tube. (It is important for the solutions to be at room temperature when nesslerized since increased temperature encourages precipitation of the reagent.) In a minute or two after the air bubbles have disappeared the nesslerized solutions should be crystal clear. The standard and unknown solutions are nesslerized as near together as possible and at once read in the colorimeter. Calculation of results is made in the regular way using tables or the general formula

$$\frac{\text{Reading of S}}{\text{Reading of U}} \times \frac{\text{Vol. of U}}{\text{Vol. of S}} \times \frac{\text{mg. N in c.c. of S used}}{\text{c.c. of U used}} \times 100 = \text{mg. nonprotein nitrogen per 100 c.c. of blood.}$$

Setting the standard at 20 mm. and using 3 c.c. of the standard nitrogen solution and 0.5 c.c. of blood, the above formula reduces to

$$\frac{600}{\text{Reading of U}} = \text{mg. N per 100 c.c. of blood.}$$

This modified Koch method has been checked against the Folin procedure and satisfactory agreement obtained. Most of the data given in Table I were obtained from single analyses but when check determinations were necessary the new method nearly always gave better agreement than Folin's. For highly accurate results the nitrogen in the reagents should be determined by using a urea solution (made up with 0.5 per cent glucose) of known nitrogen content, in place of the blood filtrate.

TABLE I

COMPARISON BETWEEN THE MODIFIED KOCH AND FOLIN'S METHOD

Mg. Nonprotein Nitrogen per 100 c.c. Blood

NO.	PATIENT	DIAGNOSIS	KOCH	FOLIN	DIFFERENCE	DIFFERENCE
			mg.	mg.	mg.	per cent
1	C. G.	Nephritis	42.0	43.5	−1.5	−3.5
2	G. R.	Nephritis	36.2	36.8	−0.6	−1.6
3	F. B.	Nephritis	93.5	93.9	−0.4	−0.4
4	A. P.	Nephritis	34.5	37.3	−2.8	−7.5
5	H. F.	Nephritis	53.6	56.0	−2.4	−4.3
6	G. P.	Nephritis	69.0	68.2	+0.8	+1.2
7	F. D.	Diabetes	36.4	37.5	−1.1	−2.9
8	I. G.	Diabetes	30.0	30.3	−0.3	−0.9
9	M. V.	Diabetes	36.8	37.9	−1.1	−2.9
10	E. F.	Diabetes	42.3	40.6	+1.7	+4.2
11	E. H.	Hypertension	64.5	61.2	+3.3	+5.4
12	M. H.	Surgical	35.3	34.5	+0.8	+2.3
13	Mixed	Hospital blood	63.2	64.5	−1.3	−2.0
14	Mixed	Hospital blood	29.0	28.1	+0.9	+3.2
15	Mixed	Hospital blood	30.8	31.9	−1.1	−3.4

SUMMARY

The period of digestion in Folin's original directions is excessive for practical purposes. Best results are obtained by stopping the digestion as soon as the brown appearance of the digestion mixture is replaced by a light green color.

When sulphuric acid with peroxide or persulphate is used for the digestion mixture a white or yellow precipitate of tungstic acid nearly always appears, either during digestion or when water is added. This acid precipitate is soluble in the alkaline Nessler's solution but the reagent itself is affected during the process of solution.

A practical modified Koch method for blood nonprotein nitrogen is described. The new method was checked against the Folin procedure and found to be accurate. This method gives promise of being the most satisfactory one available for routine use in the clinical laboratory.

The author takes pleasure in acknowledging his obligation to Dr. Arthur Knudson for assistance with the manuscript, and also to Miss Mary L. Blackmer for trying out the method in the Albany Hospital Laboratory.

REFERENCES

1. Folin, O., and Wu, H. A System of Blood Analysis, J. Biol. Chem. 38: 81, 1919.
2. Koch, F. C., and McMeekin, T. L.: A New Direct Nesslerization Micro Kjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia, J. Am. Chem. Soc. 46: 2066, 1924.

3. Wong, S. Y.: The Use of Persulfate in the Estimation of Nitrogen by Folin's Direct Nesslerization Method, J. Biol. Chem. **55**: 431, 1923.
4. Morley, A. M.: The Behavior of the Tungstic Acids Toward Sodium Hydroxide, J. Physiol. Chem. **36**: 1655, 1932.
5. Hiller, A., and Van Slyke, D. D.: A Study of Certain Protein Precipitants, J. Biol. Chem. **53**: 253, 1922.
6. Folin, O.: Supplementary Note on the New Ferricyanide Method for Blood Sugar, J. Biol. Chem. **81**: 231, 1929.
7. Looney, J. M.: The Determination of Blood Urea Nitrogen by Direct Nesslerization, J. Biol. Chem. **88**: 189, 1930.
8. Folin, O., and Svedberg, A.: Micro Methods for the Determination of Non-protein Nitrogen, Urea, Uric Acid and Sugar in Unlaked Blood, J. Biol. Chem. **88**: 77, 1930.
9. Hubbard, R. S.: The Determination of Blood Proteins by a Direct Micro Kjeldahl Method, J. LAB. & CLIN. MED. **16**: 500, 1931.

A MICRO-KJELDAHL STILL*

THOMAS P. NASH, JR., PH.D., MEMPHIS, TENN.

WHILE various procedures have been proposed for the direct nesslerization of Kjeldahl digests in the determination of nonprotein and protein nitrogen, preliminary distillation of the digests continues to be favored by many workers. For such distillations the still described herein has proved, through several years' experience in this laboratory not only in students' use but in more exacting requirements, exceptionally effective and convenient.

The various parts of the apparatus (Fig. 1) may be assembled readily in any laboratory. An iron stand (No. C) with heavy tripod base supports the entire apparatus. The iron rod is slightly loosened in the base so that all parts attached to the rod may be rotated simultaneously without altering their relative positions. Receiving vessel and micro-burner are supported upon adjustable wooden platforms. The condenser† has a straight inner tube of Pyrex glass of such diameter as will be admitted by a 50 c.c. volumetric flask; the jacket may be cut from a 100 c.c. graduated cylinder or other tubing large enough to carry 2-hole rubber stoppers with water inlet and outflow tubes. A trap between condenser and distilling tube is made from a discarded 25 c.c. pipette. Burner and condenser tubings may be passed through the crevices between the iron rod and the several attached clamps.

With the still rotated to the right there must be sufficient clearance between the table and the end of the condenser tube to admit the receiving vessel. The latter is now raised and the still is rotated to the left until the vessel sits upon the wooden platform at such level that the tip of the condenser tube dips into the receiving acid.

Where the amount of nitrogen for digestion does not require more than 1 to 2 c.c. of H_2SO_4 the digestion is carried out in 25 × 200 mm. Pyrex test tubes on a microdigestion rack. As much as 30 or 40 c.c. of fluid may be boiled down safely and without attention in such a tube if a pinch of talcum powder is

*Department of Chemistry, College of Medicine, University of Tennessee.
Received for publication, November 11, 1933.
†A West type condenser is to be preferred.

added. When the residue begins to char a small funnel with sealed stem is placed in the mouth of the tube; this prolongs the time required for clearing but avoids loss by spattering. When digestion is complete, the funnel is washed down by 10 to 15 c.c. of water, a little more talcum is added, and sufficient saturated NaOH. The total volume for distillation should not be more than 20 to 25 c.c. The tube is attached to the still, shaken to mix the contents, and clamped in position. Heating is begun and continued at the top of the liquid

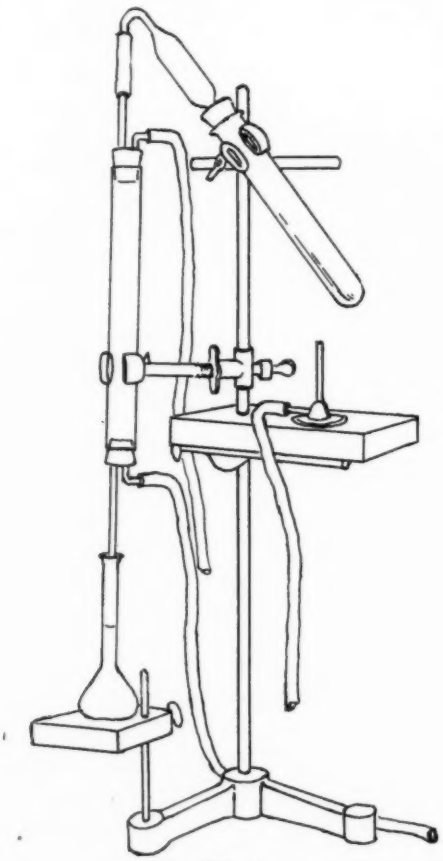


Fig. 1.

column, the burner being moved back toward the lower end of the tube as distillation proceeds. Because of the small total volume displacement of the entire system it is rarely necessary to distill over more than 10 c.c. to assure complete transfer of such quantities of nitrogen as should be employed. Before the bumping stage is reached in the distillation the still is rotated to the right so that the receiving vessel may sit upon the table and the condenser tube be out of contact with the receiving fluid. After some further distillation in this position the condenser tube is washed down. By removing the flame some of the wash water is drawn well up into the condenser tube and then expelled by a momentary reheating of the distillation tube.

The transferred ammonia may, of course, be estimated either colorimetrically or titrimetrically. We commonly employ this still for quantities of nitrogen ranging between 0.1 and 2.0 mg., and it is our experience that the step of transfer alone of such quantities does not introduce into the final value a measurable error.

A STUDY OF THE KOLMER, KAHN, AND KLINE TESTS WITH SPINAL FLUIDS*

LUCY S. HEATHMAN, PH.D., AND MARGARET HIGGINBOTHAM, B.A.,
MINNEAPOLIS, MINN.

IN 1931 Kline and Rein[1] published a microscopic slide precipitation test for spinal fluid with 1006 specimens, comparing it with a somewhat lesser number of specimens, using the Mt. Sinai Hospital Wassermann reaction and an outside Wassermann test. They concluded that the slide test was somewhat more sensitive than the Wassermann test with the same antigen, and had the advantage of being much simpler in that it required no titrated hemolytic system. It had the advantage over the Kahn of not requiring a concentration of globulins. In 1932 Eller[2] and Rein compared the Kline microscopic precipitation test on 500 spinal fluids with the Wassermann test (ice box fixation), the Ishii modified Kahn test, the albumin globulin ratio, and the colloidal gold reaction. They concluded that the slide test was more sensitive than any of these tests and gave results which agreed with clinical findings more frequently than did the other reactions.

The purpose of the present study was to compare the Kline and Kahn precipitation tests with the Kolmer-Wassermann in order to ascertain which one of the precipitation tests might be used to greater advantage as a routine test with the Kolmer-Wassermann reaction in spinal fluids. The Kahn, as mentioned earlier, has the disadvantage of the time-consuming process of concentration of globulins and of requiring much more spinal fluid than does the Kline. However, the problem immediately presented itself regarding the Kline test, as to whether this reaction would prove practical in a State Laboratory where all the specimens are shipped in, because of the fact that according to Kline the test cannot be carried out on fluids giving a negative reaction for sugar. It was soon evident that many of the specimens received in our Laboratories were negative for sugar as indicated by the Benedict test. The question of the disappearance of sugar from spinal fluid is apparently still controversial. Levinson[3] states that "the decrease in the cerebrospinal fluid sugar is probably due to the lytic action of the cells and organisms present (P188)." He also states: "Many normal fluids do not contain sufficient sugar to be reduced by Fehling's or Benedict's solution (P258)." Chevassut,[4]

*From the Minnesota Department of Health, Division of Preventable Diseases.
Received for publication, December 6, 1932.

in a series of more than 200 spinal fluids, showed the fluids contained a specific glycolytic ferment, and that the sugar disappeared in a few days even when the fluid was kept under sterile conditions, but this change was accelerated most at 37° and was inhibited at 0° C. On the other hand, Nielson[5] believes that the glycolytic process is due entirely to exogenous factors. He found glycolysis to vary greatly in spinal fluids, even sterile ones, in some beginning one hour after removal, and others not for thirty-two days. Staphylococcic growth in the fluid did not influence the sugar content, but glucose fermenting organisms did. Furthermore, he found that leucocytes or temperature did not influence glycolysis.

In our series we have grouped the specimens under sugar reaction (1) positive, (2) negative, or (3) insufficient material to carry out the sugar test. The specimens classified under treated syphilis are from syphilitic patients in whom central nervous system syphilis had been definitely diagnosed, but includes some in which it was merely suspected. The untreated ones include specimens from patients in whom systemic syphilis had been diagnosed and previously treated, central nervous system syphilis now being suspected. Those unclassified include specimens in which no definite history could be obtained. It is regretted that more complete histories could not be procured to permit of a more detailed description. It will be noted by referring to Tables I and II, that while 1632 tests were carried out with the Kolmer and Kline, there were only 695 of these specimens in which the Kahn test was done.

TECHNIC

The technic as published by Kline[1] for spinal fluids was followed with the exception that the antigen was made according to his 1931[6] modification. We have found it necessary to titrate the Kline antigen, as different lots may vary considerably. New antigen was standardized to conform with the former lot of our antigen used, and with antigen furnished us by Kline. The standardization was accomplished by diluting the antigen with varying amounts of cholesterinized alcohol until uniform results were obtained, using the various antigens with at least 100 different sera of varying degrees of positivity as well as negative ones. Kline's technic was also modified in that spinal fluids giving negative reactions for sugar were used in the test. A large majority of these fluids were clear; only very rarely were there heavily contaminated ones. Fluids were also tested in which there was insufficient material to carry out the Benedict test for sugar. The Kolmer[7] technic was strictly followed. The Kahn[8] technic was used as given by him in 1929. All tests were read by two serologists and there was little variation in the readings. In this study we have considered 2+ to 4+ as positive in any test, although Kolmer considers a + as positive. Kline's method of reading his test is as follows: "± to 4+ any test in syphilitic serum equals agreement," which implies that a ± in a syphilitic serum is considered positive. He further states that: "2+ to 4+ any test in nonsyphilitic serum equals false positive."

RESULTS

In Table I is shown the agreement and disagreement of the Kolmer-Kline, Kolmer-Kahn, and Kahn-Kline tests respectively, according to the sugar

TABLE I

SHOWING AGREEMENT OF KOLMER, KAHN, AND KLINE

	SUGAR	TREATED SYPHILIS (C. N. S.)			UNTREATED SYPHILIS			NO HISTORY OF SYPHILIS			UNCLASSIFIED			ALL GROUPS		
		TOTAL SPEC.	AGREEMENT SPEC.	%	TOTAL SPEC.	AGREEMENT SPEC.	%	TOTAL SPEC.	AGREEMENT SPEC.	%	TOTAL SPEC.	AGREEMENT SPEC.	%	TOTAL SPEC.	AGREEMENT SPEC.	%
Kolmer-Kline	−	35	28	80.6	38	35	92.0	163	154	94.4	299	281	94.0	535	498	93.0
	+	55	52	94.5	68	61	89.7	312	310	99.3	379	340	94.7	814	763	93.6
	0	37	30	81.0	11	9	81.8	87	83	95.4	148	135	91.2	283	257	91.0
Total		127	110	86.6	117	105	89.7	562	547	97.3	826	776	93.9	1632	1518	93.0
Kolmer-Kahn	−	14	13	92.8	22	20	90.9	81	81	100.0	83	75	90.3	200	189	94.5
	+	30	26	86.6	46	39	82.6	208	208	100.0	174	155	89.0	458	427	93.2
	0	1			2	1*		19	18	94.7	15	8*	53.0	37	27	73.0
Total		45	39	86.6	70	60	85.7	308	307	99.6	272	238	87.5	695	643	92.5
Kahn-Kline	−	14	13	92.8	22	20	90.9	81	81	100.0	83	76	90.0	200	190	94.5
	+	30	27	87.0	46	41	93.0	208	207	99.5	174	154	90.0	458	429	94.0
	0	1			2			19	19	100.0	15	13	76.4	31	32	87.0
Total		45	40	86.6	70	61	87.1	308	307	99.6	272	243	89.3	695	651	93.6
All three	−	14	10	71.4	22	20	90.9	81	81	100.0	83	75	90.3	200	186	90.3
	+	30	26	86.6	46	39	82.6	208	207	99.5	174	154	90.0	458	426	93.0
	0	1			2			19	18	94.7	15	8*	53.0	37	26	70.0
Total†		45	36	80.0	70	59	84.2	308	306	99.6	272	237	87.1	695	638	91.8

2+ to 4+ considered positive in all cases.

Specimens noted as agreement are comprised of the following:

All three tests negative, −; all three tests ± +; all three tests 2+ to 4+.

Percentages are compiled according to the agreement of specimens with sugar reaction positive or negative according to Benedict, or insufficient material to carry out the sugar test. The final percentage in each classification is compiled for the entire group regardless of sugar.

*Too small for single group compilation but included in totals.

†Includes only 695 specimens from Kolmer-Kline group.

reaction, and in those in which there was not sufficient sugar to run the Benedict test. Although the percentage of agreement is variable for the several groups, the figures demonstrate no appreciable difference in agreement for specimens with or without sugar, respectively. In the total agreement the Kahn-Kline agreement is slightly higher (93.6 per cent) than in the Kolmer-Kline (93.0 per cent) or the Kolmer-Kahn (92.6 per cent). The figures are but a little higher than that of Kline[1] for his slide test and the Mt. Sinai Hospital Wassermann test (92.3 per cent) in which all specimens contained sugar. The agreement obtained by Eller and Rein[2] with the diagnostic Kline test and the noncholesterinized Wassermann test in a combined group of proved neurosyphilitics and nonneurosyphilitics was 93.0 per cent, while their agreement in patients with no evidence of neurosyphilis was 99.59 per cent as compared to 97.3 per cent Kolmer-Kline agreement in our series in patients with no evidence of syphilis. Table I shows the percentage of agreement of the Kolmer, Kahn, and Kline with respect to positive and negative sugar reactions. This Table demonstrates that the agreement between tests is very close, irrespective of sugar content. The discrepancies which occur might possibly be explained by the difference in numbers of specimens in the two groups based on the Benedict test. Statistical calculation has demonstrated no significant difference in any of these figures. Furthermore, statistical calculation has shown no significant differences in results of the Kline test with and without sugar, or the Kolmer and Kahn tests with and without sugar, respectively. In Table II in which the positives for each test are given it may be seen that the total percentages obtained for the Kolmer and Kline are identical, while the Kahn is slightly lower. Eller and Rein reported the Kline diagnostic test more sensitive than the Kahn. In our series the Kahn was more sensitive in treated cases, but the Kline more sensitive in untreated ones.

In a few instances only were we able to carry out repeated Kline and sugar tests. Of 71 spinal fluids which gave Kline reactions of + to 4+, there was no

TABLE II

SHOWING THE PER CENT POSITIVES OF THE KOLMER, KAHN AND KLINE, RESPECTIVELY

	SUGAR	TREATED SYPHILIS (C. N. S.)		UNTREATED SYPHILIS		NO HISTORY OF SYPHILIS		UNCLASSIFIED		TOTAL	
		%	NO.	%	NO.	%	NO.	%	NO.	%	NO.
Kolmer	–	18.1	23	30.7	36	0.18	1	4.8	40		
Positive	+	22.8	29	57.2	67			5.7	47		1632
	0	12.6	16	6.8	8	0.18	1	2.2	18	17.5	
Total % & No.		53.5	127	94.8	117	0.4	562	12.7	826		
Kahn	–	22.2	10	28.5	20	0.6	2	3.7	10		
Positive	+	31.1	14	55.7	39			6.2	17		695
	0	1.2	1	1.4	1			1.10	3	16.5	
Total % & No.		54.5	45	85.7	70	0.6	308	11.0	272		
Kline	–	13.3	17	30.7	36	0.7	4	4.6	38	115	
Positive	+	20.4	26	52.9	62	0.3	2	6.6	55		1632
	0	11.0	14	8.5	10			2.6	22	17.5	
Total % & No.		49.9	127	92.3	117	1.1	562	13.9	826		

change in this test in 52 (73.2 per cent) after an interval of two to nineteen days, regardless of the sugar test being negative later in all. In 10 of these after inoculation of *B. coli* the sugar test became negative but there was no change in the Kline. On a second examination in 13 (18.3 per cent) of the 71, the reaction was less marked; i.e., 7 giving 4+ became 2+; 5, 4+ became +; and one, 2+ became +. From a case giving a 4+, one specimen (1.4 per cent) became negative in five days. Four (5.6 per cent) giving a 2+ became negative in eight to nineteen days and one specimen (1.4 per cent) giving a + became negative in four days. In 14 specimens which had no sugar on the first examination, there was no change in + to 4+ Kline reactions.

CONCLUSIONS

In the series of tests carried out here the relation between the results of complement fixation reactions and the precipitation tests was very close in the groups containing sugar and the ones in which sugar was absent. Judging by our figures it would appear feasible to perform Kline tests on spinal fluid specimens in which the presence of sugar cannot be demonstrated, providing the fluids are not grossly contaminated. However, it is shown that in a few specimens in which repeated Kline tests were done, a change occurred in some. Whether this was due to the factor causing the disappearance of sugar is a point to be determined. It seems logical that if a specimen is suitable for the complement fixation test, it should be possible to carry out a precipitation test, since it is a well-known fact that the substance which is responsible for the fixation of complement is responsible for the formation of the precipitate. On the other hand, complement fixation and precipitation have quite different optimal conditions. However, judging by the close agreement of our results of complement fixation and precipitation on fluids with and without sugar, it does not appear that the presence or absence of sugar is a factor. In the study made here the Kline test seemed to be preferable to the Kahn as a routine test with the Kolmer, since it gave a higher percentage of positives than the Kahn in untreated clinically syphilitic cases, while the Kahn offered no significant advantage over the Kolmer in treated cases. The performance of the Kline being more expeditious than the Kahn and being more easily read, offers an advantage over the Kahn. However, the Kolmer test probably due to the fact that it is an ice box fixation test and, therefore, usually more sensitive in spinal fluids where often a small amount of reagin is present, generally appears to be superior to either precipitation test.

REFERENCES

1. Kline, B. S., and Rein, C. R.: A Microscopic Slide Precipitation Test for Syphilis with Spinal Fluid, J. Lab. & Clin. Med. **16**: 399, 1931.
2. Eller, J. J., and Rein, C. R.: The Microscopic Slide Precipitation Test for Syphilis with Spinal Fluid, N. Y. State J. Med. **32**: 71, 1932.
3. Levinson, A.: Cerebrospinal Fluid in Health and Disease, ed. 3, 1929, The C. V. Mosby Company, p. 188, and p. 258.
4. Chevassut, K.: Glycolysis in Cerebrospinal Fluid, Quart. J. Med. **11**: 91, 1927.
5. Nielson, O. T.: Untersuchungen über Glykolyse in Spinal-flüssigkeiten, Zeitschrift für die Gesamte Experimentelle Medizin **64**: 522, 1928.
6. Kline, B. S.: Microscopic Slide Precipitation Tests for the Diagnosis and Exclusion of Syphilis, Brit. J. Ven. Dis. **7**: 32, 1931.
7. Kolmer, J. A.: Serum Diagnosis by Complement Fixation, Philadelphia, 1928, Lea & Febiger.
8. Kahn, R. L.: Am. J. Syph. **13**: 557, 1929.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PREGNANCY, Simple Test for, Using Immature Female Albino Rats, Kelly, G. L. J. A. M. A. **100**: 1010, 1933.

Intraperitoneal injections of from 5 to 15 c.c. of urine from pregnant women will cause premature establishment of the vaginal orifice in the albino rat weighing from 30 to 40 gm., usually within from seventy-two to eighty-four hours.

Intraperitoneal injections of the same amount of urine from nonpregnant women will not cause such premature opening of the vagina.

Two animals should be used and the opening of the vaginas of both is read as positive; of one, as doubtful (the test should be repeated); of neither within ninety-six hours, as negative.

If the vagina is open and the orifice obscure, one may see it by holding the animal by the neck and shoulder region and pulling strongly on the base of the tail. In this maneuver the abdomen should not be compressed.

The main advantages are the simplicity of the test and the ease of reading the result.

TUBERCULIN, Comparative Sensitiveness of the Pirquet and Intracutaneous Test, Aronson, J. D., Zacks, D., and Poutas, J. J. Am. Rev. Tuber. **27**: 465, 1933.

It was shown in a comparative study that the intracutaneous tuberculin test is more sensitive than the Pirquet test in determining the incidence of tuberculous infection, and that the results obtained by the two methods are not directly comparable.

TRANSFUSION: Technic for Direct Matching of Blood, Simpson, J. C. Am. J. Clin. Path. **3**: 256, 1933.

1. Draw a drop of blood from patient's finger tip to the 1 mark in a leukocyte counting pipette and follow with 5 per cent sodium citrate solution to the 11 mark.
2. Mix thoroughly by shaking and transfer to small glass vial suitably marked for identification.
3. Repeat for each donor.
4. With an erythrocyte pipette, make a 9 to 1 combination of patient's and donor's blood by drawing blood from donor's vial to the first or 0.1 mark and patient's to the 1 mark at top of lower portion of the pipette.
5. Transfer mixture to end of a glass slide and mix by alternate sucking and blowing three or four times. Add a small drop of saline and cover with cover slip.
6. Now make a 1:1 dilution by drawing patient's blood from the vial to the 0.5 mark and donor's blood to the 1 mark. Mix and transfer to the other end of slide in same manner.
7. Prepare a separate slide in this way for each donor. There is then a mixture of 9 parts of patient's blood to 1 of donor's at one end of slide and a 1 to 1 mixture at other end.
8. Agglutination will show up both micro- and macroscopically in two or three minutes at room temperature.
9. If no clumping occurs in fifteen minutes transfusion is safe.
10. Transfusion is dangerous if agglutination is present in the 9 to 1 combination.

11. If the 9 to 1 mixture is clear and the 1 to 1 shows agglutination, transfusion can be done but with caution; be ready to stop at once if untoward symptoms develop.

TISSUE: The Adaptation of Formalin-Fixed Tissue for Mallory's & Wright's Staining Methods, Kernohan, J. W. Am. J. Clin. Path. 3: 255, 1933.

The formalin-fixed material is washed briefly in running water or ammonia water (10 c.c. of tap water and approximately 60 drops of ammonia), although both these procedures may be omitted. If there is much blood in the tissue, the ammonia water reduces the formalin precipitate on the stained preparations. The tissue is fixed in Zenker's solution for six to eight hours, and washed in running water for twenty minutes to half an hour. The tissue is placed overnight or longer in Weigert's secondary mordant for myelin sheath stains. It is then embedded in paraffin and cut and stained in the usual way with any of Mallory's stains.

SCARLET FEVER: The Addis Sediment Count In, Lyttle, J. D. J. Clin. Invest. 12: 95, 1933.

The technic of Addis for the quantitative mensuration of protein, casts and cells in the urine has been applied to 14 cases of scarlet fever. All cases showed moderate transient increases in the excretion of protein and formed elements during the period from eight to forty-five days after onset of scarlet fever.

URINE: The Addis Sediment Count in Normal Children, Lyttle, J. D. J. Clin. Invest. 12: 87, 1933.

The following is suggested as a tentative standard in normal children four to twelve years old:

Protein	35 mg.
Casts	10,000
Red blood cells	600,000
Epithelial and white blood cells	600,000 (male)
	1,000,000 (female)

TUBERCULOSIS: Studies in the Pathogenesis of Primary. II: Tendencies in Adult Tuberculous Infection, Sweany, H. C., Am. Rev. Tuber. 27: 575, 1933.

A report has been made of 6 primary-like infections in adult individuals that reveal a difference from the usual type in that the lymph node complex was very small or entirely lacking. The variation is so constant in adult people living in civilized communities that it raises the question of whether it is not the rule in such individuals. A few hints are made toward a more rational pathologic classification of primary tuberculous lesions than is represented by existing theories.

TUBERCULOSIS: Studies on the Pathogenesis of Primary. I: The Regressive Lesion, Sweany, H. C. Am. Rev. Tuberc. 27: 559, 1933.

A study of the regression of the primary tubercle has been made, based on the findings in 188 pairs of lungs removed at necropsy, radiographed, and sectioned by a special method. The incidence and location of these lesions were found in keeping with the ideas of Kuss, Ghon and others, but there was a higher incidence of what appeared to be multiple primary residues. This was thought to be due to a more certain method of localization. Of 88 specimens of characteristic primary lesions 76 were in the parenchyma and 65 in the lymphatics. Twenty-three were located only in the parenchyma and 11 only in the lymph nodes.

In 84 calcified parenchymal lesions bone was found in 22; while in 97 lymph nodes bone was found in 23, revealing practically no difference in bone formation between the local and lymphatic lesion and an incidence of 25.1 per cent of all without the usual lymph node exaggeration. The mechanism of calcification, ossification and resorption are described.

The question arose regarding the possibility of the liberation of tubercle bacilli as a result of the resorption of the walls of the primary lesion, because walls were entirely gone in more relatively young individuals (one seventeen years old) while bacilli were living in most large primary lesions in people under twenty-five years of age.

Approximately half of the calcified parenchymal lesions may be seen on antemortem radiographs, for, out of 98 showing on the postmortem pictures, only 48 could be seen on the antemortem films. Ten contained very small primary lesions and thirty none at all. Many of these two groups showed a modified type of primary lesion, indicating that there had been only a partial sensitizing effect from the first infection. Six appeared to be adult primary lesions.

WIDAL REACTION: The Bacteriology and Immunology of Enteric Fevers, Pijper, A. South African M. J. 8: 283, 1933.

The author relies upon the complement fixation reaction for the early recognition of typhoid fever in preference to the Widal reaction the shortcomings of which he discusses.

He calls attention to the presence of and difference between H (flaky) agglutinins and O (dense, compact flakes) agglutinins and their differing clinical significance and also to the fact that O agglutinins can only be demonstrated when agglutination tests are made upon living organisms or upon alcoholic suspensions.

He formulates the following rules:

1. The Widal reaction, as commonly done with killed suspensions, elicits only H agglutinins and does not demonstrate O agglutinins.

2. While typhoid patients *may* produce both H and O agglutinins, they all produce O agglutinins early in the disease. In many cases H agglutinins may never be produced or too late to be of diagnostic value.

3. O antibodies are the same antibodies as those producing complement fixation, hence either test may be used for their detection providing that the agglutination test is carried out in accordance with the dicta above presented.

4. Typhoid vaccination causes the production of H agglutinins which may show a non-specific increase in the presence of febrile reactions whereas O agglutinins increase in titre only when the patient has typhoid.

5. H antibodies are valueless as a means of defense. O antibodies are the real immune bodies.

From these premises the following deductions follow:

a. The serologic diagnosis of typhoid depends upon the demonstration of O antibodies by complement fixation or suitable agglutination tests.

b. The strength of the positive reaction, as it depends upon the presence of truly defensive antibodies, is of some prognostic value. In this connection the test is best correlated with the blood culture.

c. Carriers frequently do not show H agglutinins but always show O agglutinins and may be thus recognized.

d. Oral immunization (Besredka method) causes the production of O antibodies.

DIPHTHERIA: Replacement of Toxin-Antitoxin by Toxoid With a Consideration of the Comparative Dosage, Park, W. H. Am. J. Pub. Health 23: 600, 1933.

The conclusions advanced follow:

The manufacturers should be required to state on their labels the number of flocculation (antigenic) units in the amount in the syringe or vial, or the number of units per cubic centimeter.

Two doses of from 5 to 10 antigenic units each are sufficient to immunize from 90 per cent to 98 per cent of young children, and of a somewhat less percentage of older children. Even 2 doses of 2.5 units each will give us as much immunity as 3 doses of 1 c.c. of standard toxin-antitoxin. If by refining we are able to obtain a much stronger toxin which does not produce too much local irritation we may use one dose only.

Toxoid has an advantage over toxin-antitoxin in being much more stable.

An interval of two weeks, or longer, is advisable between injections, but where it is much more convenient to make the interval only a week it is proper to do so.

At any age after three months, infants respond well to injections of toxoid. The choice time in the country is probably at six months, or a little later; and in the city, nine months or a little earlier. The difference in the most desirable age is due to the fact that in the country a smaller percentage of infants obtain passive immunity from the mothers than in the city.

RHEUMATIC FEVER, Blood Cultures in Children, Wilson, M. G., and Edmond, H. Am. J. Dis. Child. **45**: 1237, 1933.

A total of 536 blood cultures were sent to the laboratory for study. One hundred and forty-seven (26 per cent) were found to be contaminated (137 on the first and 10 on subsequent openings) and were therefore discarded. Of 389 blood cultures, 293 (75 per cent) remained sterile for the four-week period of study. Ninety-six (25 per cent) of the cultures were considered positive.

Thirty-two of the uninoculated cultures were sent to the laboratory (unidentified) and subjected to identical bacteriologic examination. Two, or 6 per cent, were found to be contaminated and were discarded. Ninety-four per cent remained sterile throughout the period of study.

Two hundred and thirty-six blood cultures from 67 children with rheumatic disease were studied. In 46 per cent of these children, positive cultures were secured. Positive cultures were obtained in 37 per cent during the active stage and in 41 per cent during an apparently inactive period.

In the control series (which included normal infants and children as well as those who were ill or were convalescing from various infections), 153 blood cultures from 78 children and 13 adults were studied. Forty-one per cent of these cultures were positive. Positive cultures were secured in 33 per cent of the healthy infants and children of this control group.

The microorganisms recovered from all of the blood cultures were of 3 types, Streptococcus viridans, Streptococcus anhemolyticus and pleomorphic bacilli. About one-half of the organisms recovered were streptococci; the remainder were pleomorphic bacilli.

The incidence of recovery of these organisms from both the rheumatic and the control series were comparable.

The incubation period varied from three to thirty-two days. About one half of the strains (streptococci and pleomorphic bacilli) were recovered within a two-week period of incubation.

SPIROCHETA PALLIDA, Modification of Dieterle Method for Single Sections, Krajian, A. A. Am. J. Syph. **17**: 127, 1933.

1. Fresh blocks of tissue about 5 mm. thick are fixed in 10 per cent formalin at 70° C. for ten minutes. Of course, formol fixed tissue does not require this.
2. Cut thin sections (5 to 7 microns) on the freezing microtome.
3. Place the sections in the following solution for fifteen minutes at 70° C.

Uranium nitrate	1.0 gm.
Formic acid, C. P. 85 per cent	3.0 c.c.
Glycerine	5.0 c.c.
Acetone	10.0 c.c.
Alcohol, 95 per cent	10.0 c.c.

4. Wash for a few seconds in distilled water.
5. Place sections in a 0.75 per cent aqueous solution of silver nitrate for one hour at 70° C.
6. Wash in distilled water for a few seconds.

7. Place sections in 10 c.c. of the following developing solution for five to ten minutes, to which, just prior to use, one drop of albumin glycerin fixative is added and thoroughly mixed. The egg albumen glycerin fixative is that commonly used to fix paraffin sections to slides.

Hydroquinone	0.62 gm.
Sodium sulphite	0.12 gm.
Acetone	5.0 c.c.
Neutral formaldehyde, C. P. 40 per cent	5.0 c.c.
Pyridine	5.0 c.c.
Saturated solution of gum mastic in 95 per cent alcohol	5.0 c.c.
Distilled water	30.00 c.c.

8. Wash for a few seconds in distilled water.

9. Draw sections onto slides, dehydrate with absolute alcohol, clear in xylol, and mount in xylol-gum damar.

PERTUSSIS, Medium for Isolation, Bailey, J. H. J. Infect. Dis. 52: 96, 1933.

The ingredients are: potato 200 gm.; potato starch, 18 gm.; chemically pure glycerin, 30 c.c., and distilled water, 600 c.c.

The potatoes are pared and cut into thin slices. A quantity of potato slices weighing 200 gm. is placed in a tared double boiler along with the potato starch, glycerin and water. Potato flour may be used in place of the potato starch. The mass is then cooked for two hours, being stirred constantly until it thickens, then only occasionally. Thickening usually occurs within ten minutes. At the end of two hours the water lost by evaporation is replaced, and to each 1 c.c. of starch mass is added 3 c.c. of 0.6 per cent sodium chloride solution. The mixture is shaken thoroughly and then filtered through four layers of cheesecloth. This filtration is rapid. The filtrate is then distributed, in 200 c.c. lots, into flasks of convenient size (500 to 750 c.c.) and 10 gm. of agar added to each flask. The flasks are plugged and sterilized in the autoclave of twenty minutes at 15 pounds (6.8 kg.) of pressure. This forms the stock base of the medium, and may be stored in the refrigerator until needed. To complete the medium, a flask of the base is melted, preferably in the autoclave, and when the melted medium has cooled to about 40° C., 200 c.c. of sterile defibrinated horse blood is added and thoroughly mixed. Then 6 c.c. of sterile half-normal lactic acid is added and thoroughly mixed, care being used to prevent frothing of the medium. The medium may now be poured into petri dishes or tubed.

A medium for the isolation of *B. pertussis* should be a bright red to give optimum results. This fact has not received the emphasis due it.

SYPHILIS, Comparison of Results Obtained With Kahn, Kline, Hinton, Meinicke, Sachs-Georgi, and Rosenthal Tests for, Burdon, K. L., and Duggan, L. B. Am. J. Syph. 17: 110, 1933.

The results of Kline, Kahn, Hinton, Sachs-Georgi, Meinicke, and Rosenthal tests performed at the same time on the sera from 400 individuals indicate that the Kline, Rosenthal, and Meinicke methods are the most sensitive, that the Hinton and Kahn tests are somewhat less so, and that the Sachs-Georgi test is comparatively insensitive. With the sera from 80 patients with late or latent syphilis the Sachs-Georgi method was positive in no more than 6 per cent, while positive reactions were recorded with the Kahn test in 24 per cent, the Hinton in 28 per cent, the Kline in 56 per cent, the Meinicke in 58 per cent, and the Rosenthal in 61 per cent.

Comparison of the results of parallel tests by the Kline and Kahn methods on 1235 specimens confirmed the greater sensitivity of the Kline over the standard Kahn test. The latter gave a reaction of slightly greater degree in only 7 cases (0.5 per cent). On the other hand, in approximately 14 per cent of the tests the Kline reaction was doubtful (± or +) while the Kahn was negative, or definitely positive (++, +++, or ++++) while

the Kahn was doubtful, and in another 3.6 per cent the Kline test was positive and the Kahn entirely negative.

In the great majority of instances the reactions of the more sensitive tests were justified by the known existence of syphilitic infection in the patients. False positive reactions were not observed with any of the methods except with the Kline test, and then only in 2 instances, when the "very sensitive" antigen emulsion was used. A few nonspecific doubtful (+ or –) reactions in normal sera were recorded, however, with all of the methods, except the Sachs-Georgi test. These reactions were observed most frequently with the Rosenthal test, but the number recorded rapidly declined as experience with the method increased. The next largest number were observed with the Kline test, doubtful reactions being recorded in about 3 per cent of the 227 normal sera tested. Less than 1 per cent of the Kahn, Hinton, and Meinicke tests gave these reactions.

Since all of the tests have been developed empirically, and none are, in a strict sense, specific for syphilis, there is no absolute standard by which their merits may be judged. They are all more or less efficient indicators of the same change in the serum which is the characteristic sign of syphilitic infection. The methods differ considerably in sensitivity when a certain particular technic prescribed by their originators is followed, as in the present series of tests. It must be realized, however, that the degree of sensitivity shown by a certain method in such a comparative study is not a fixed or constant attribute. Without any fundamental changes in the technic any one of the methods may be adjusted as to be more or less sensitive. It may be remarked parenthetically that the sensitivity of complement fixation tests is also subject to wide variation, and the statements sometimes seen in the literature to the effect that this or that precipitation test is more or less sensitive than "the" Wassermann test are utterly pointless. Among the precipitation tests the most useful are those which are most readily adjusted to different degrees of delicacy, and which give clear-cut reactions, easily controlled, throughout a wide range of sensitivity. With such a test, in the hands of a competent serologist who understands thoroughly the factors influencing that particular method, a really adequate serologic study of cases showing borderline reactions could be made, and these cases constitute the only real problem. Economy of time and materials and ease of preparing reagents are further qualities to be desired. Thus, in appraising the various tests, their general practicability is after all a primary consideration.

With this in mind the authors believe the Kline and Kahn methods are to be especially commended, and that the Hinton test is also acceptable, whereas the Sachs-Georgi and Meinicke methods are not satisfactory. When the Rosenthal test is conducted according to the new procedure described, it appears to be of practical value as a supplemental microscopic test, particularly in laboratories where Kahn tests are done routinely.

The authors' results, as well as those of many other workers, show that no two methods can be expected to yield identical reactions in all sera. Nothing is to be gained, however, by performing a multiplicity of different tests on every serum. It would seem that the most desirable arrangement would be to carry out first a simple, rapid, presumptive test (conveniently a microscopic method), so adjusted as to be highly sensitive, such as the Kline test. This would result in the elimination from further consideration of all the frankly negative specimens which commonly comprise as much as 80 per cent of all. (The use of a presumptive form of the Wassermann test for the same purpose was long ago recommended.) The same test in less sensitive form, or perhaps preferably another well understood method, either a complement fixation procedure or a test tube precipitation test, may then be performed on the remaining sera. Those specimens showing borderline reactions should be subjected to still further testing by one of the methods in quantitative form.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Asthma, Hay Fever and Related Disorders*

THIS is a very readable primer on allergy for the use of the patient, a perusal of which will give him an understanding of how allergy behaves and what the allergist is attempting to accomplish. It is not a guide to treatment in that there are no detailed instructions to be used in this or that case. It is, in effect, just what the author intended, an explanatory handbook of just what we are talking about when we discuss allergy.

Diseases of the Heart†

SINCE Sir Thomas Lewis' preeminence as a cardiologist is known by all, the value of the book under review may be taken for granted. Those who have read his earlier volumes realize the conciseness of his manner of presentation, the absence of paraphrasing and superfluity.

The present volume to an extent represents a milestone in the published volumes of Sir Thomas Lewis. First, his style while in no sense verbose is freer and more elastic. Second, there is a minimum of presentation of protocols and experimental observation. Third, and this is the real reason, he is writing the book for the general practitioner of medicine. It is not a report of experimental studies but a reference volume on the treatment of the general run of cardiac diseases. The reader will not be baffled by finding that he must take an electrocardiographic tracing of each case or make special laboratory studies. These are of utmost value in cardiac research and often of great value in clinical studies, but more often entirely unnecessary.

The author's arrangement is original and most logical. The first section deals with cardiac failure in its various phases; the second with coronary thrombosis; the third with angina; the fourth with changes of rate and rhythm, and this is followed by discussions of valvular disease, effort syndrome, pulmonary edema, pericarditis, infection, hypertension, arteriosclerosis, congenital lesions, etc.

Scattered through the volume are statements which one could almost term aphorisms which the author has derived from his own experience and which we almost wish he had italicized. The following are examples.

"Cardiac failure may be divided into two stages; the stage of symptoms or waning reserve and the stage of signs or of circulartory embarrassment and breakdown."

"It is to be realized that by the time congestion of the venous system sets in, nine-tenths of the heart's capacity to perform its task has been lost."

"Of the very numerous tests of cardiac efficiency . . . there is none that approaches in delicacy the symptom of breathlessness."

"It is generally redundant to set up deliberate exercise tests."

---

*Asthma, Hay Fever and Related Disorders. A Guide for Patients. By Samuel M. Feinberg, M.D., F.A.C.P., Assistant Professor of Medicine and Attending Physician in Asthma and Hay Fever Clinic, Northwestern University Medical School; Attending Physician, Cook County Hospital, Chicago. Cloth, pages 124, illustrated. Lea and Febiger, Philadelphia, 1933.

†Diseases of the Heart. Described for Practitioners and Students. By Sir Thomas Lewis, C.B.E., F.R.S., M.D., D.Sc., LL.D., F.R.C.P., Hon. D.Sc. (Michigan); Physician in charge of Department of Clinical Research, University College Hospital, London; Physician of the Staff of the Medical Research Council; Physician in chief (pro tem.) Peter Bent Brigham Hospital, Boston; Honorary Fellow New York Academy of Medicine; Corresponding Member Association of American Physicians and Interstate Postgraduate Medical Association. Cloth, pages 297. The Macmillan Company, New York, 1933.

"A comparison between a healthy and an unhealthy individual is usually far less satisfactory than between the healthy and unhealthy states of the same subject."

"In studying cardiac failure at certain stages, the signs to watch are not in the heart, but in the veins and the liver, in the legs and at the bases of the lungs."

American and Canadian Hospitals*

THIS reference volume takes its place alongside of the Directory of the American Medical Association and Fifield's "American Physicians and Surgeons." The editor has developed it with the cooperation and endorsement of the American Hospital Association and other similar interested associations. The first 80 pages contain brief historical descriptions by various contributors, of the development of the various hospital associations such as the American Hospital Association, the Canadian Hospital Council, the American College of Surgeons, the Catholic Hospital Association, the American Nurses' Association, the American Occupational Therapy Association, etc. The major portion of the 1500 pages is taken up with descriptive information of hospitals in the United States and Canada, including even such remote sections as Alaska, Guam, Hawaii, the Philippine Islands, Labrador, Newfoundland, and Yukon.

The list is not selected but includes all hospitals, even small hospitals in the smallest villages with no more than five beds. Information available includes description of types of cases admitted and not admitted, special departments, ownership, whether approved by American College of Surgeons, whether approved by the A. M. A. for interneship, whether a member of the American Hospital Association, size, cost, rates, annual statistical information on admittances, and officers.

This is a volume that should be had by all hospitals, by business enterprises trading with hospitals, and should be available for physicians to consult in selecting the proper hospital to which to send their patients.

A Standard Classified Nomenclature of Disease†

THE need for a standardized nomenclature acceptable to all physicians has been strongly felt for many years. Classifications such as those used at Bellevue Hospital, the Peter Bent Brigham and associated hospitals and others have been acceptable but have not been uniform. Since there is rather constant need for revision and since these revisions should where possible correlate the various classifications, a conference on nomenclature of disease was held in New York on March 22, 1928, upon the invitation of the New York Academy of Medicine. From this developed a large committee entitled "The National Conference on Nomenclature of Disease" headed by Haven Emerson, Henry Christian, and E. H. L. Corwin and having representatives from a large number of the leading medical societies in the country.

The outcome of the work of the conference is the present volume. It has been approved by all of the cooperating organizations and hospitals and will undoubtedly be from now on the recognized standard. The volume is not large and is so thoroughly indexed that no physician will have any difficulty in adapting his diagnoses to the standard nomenclature. The advantages of such a procedure are obvious and it is hoped that all hospitals will henceforth require that diagnoses be recorded in accordance with this nomenclature and that all physicians who make any attempt at keeping cross files by diagnosis will adopt it.

---

*American and Canadian Hospitals. A Reference Book giving Historical, Statistical and other information on the Hospitals and Allied Institutions of the United States and Possessions, and the Dominion of Canada. Edited by James Clark Fifield with the co-operation of the American Hospital Association. Cloth, pages 1560. Midwest Publishers Company, Minneapolis, 1933.

†A Standard Classified Nomenclature of Disease. Compiled by The National Conference on Nomenclature of Disease. Edited by H. B. Logie, M.D., C. M., Executive Secretary. Cloth, pages 702. The Commonwealth Fund, New York, 1933.

The Journal of Laboratory and Clinical Medicine

VOL. XVIII ST. LOUIS, MO., SEPTEMBER, 1933 NO. 12

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - - - - - - - CINCINNATI
J. J. R. MACLEOD, M.B. - - - - - ABERDEEN, SCOTLAND
W. C. MACCARTY, M.D. - - - - - - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - - - - - - COLORADO SPRINGS
VICTOR C. MYERS, PH.D. - - - - - - - - CLEVELAND
RUSSELL L. HADEN, M.D. - - - - - - - - CLEVELAND
JOHN A. KOLMER, M.D. - - - - - - - PHILADELPHIA
ROBERT A. KILDUFFE, M.D. - - - ATLANTIC CITY, N. J.
GEORGE HERRMANN, M.D. - - - - - - - - GALVESTON
T. B. MAGATH, M.D. - - - - - - - ROCHESTER, MINN.
DEAN LEWIS, M.D. - - - - - - - - - BALTIMORE
M. H. SOULE, SC.D. - - - - - - - ANN ARBOR, MICH.

Contents of this Journal Copyright, 1933, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second Class Matter.

EDITORIAL

Liver and Brain—Pathologic Interrelationships

THERE has been, shall we say, a superstition through the ages that the liver, under certain circumstances, exerts a direct action upon the mind or the brain. That the supposed action is a deleterious one is indicated by such terms of ancient usage as "choleric" and "hypochondriac." In more recent years the possibility of recognizable organic damage to the central nervous system due to or associated with liver pathology has aroused more serious interest. The most clear-cut example is the occurrence of hepatic cirrhosis with degeneration of the lenticular nucleus, described by Wilson in 1912. Many instances have since been reported of comparable coincidences in which hepatic disease preceded nervous manifestations and in which at autopsy spongy degeneration of the corpus striatum with proliferation of blood vessels and glia cells, was demonstrated. Some of these cases have presented symptoms

suggestive of Parkinson's disease, others have resembled encephalitis. Occasionally there has been an associated combined degeneration of the spinal cord.

Explanatory theories have suggested that liver disease was the primary factor, with secondary toxic action on the nervous system; or that both the hepatic and central nervous pathology were due to some intoxication from another source within the body; that the combined occurrence of disease in both tissues represented some congenital deficiency; or that the original source of trouble was in the central ganglia regulating hepatic circulation, disease of which produced functional disturbances in the liver.

Crandall and Cherry have recently observed that in individuals suffering from hepatic disease there is a decided increase in the oil-splitting lipase in the serum. This was also observed in patients suffering from multiple sclerosis. Crandall and Weil have recently reported further experimental investigations in this regard.

These authors have produced liver damage in dogs by either of three methods; ligation of the common bile duct; ligation of the pancreatic duct; and by an Eck fistula. They have also produced liver damage in rats by ligation of the common bile duct. Ligation of the pancreatic duct or bile duct produces a severe fatty degeneration of the liver. The degeneration is not as severe in rats.

The blood serum of dogs with liver damage produced by these methods contains a substance, appearing on and after the fourth day, which exerts a destructive action on the spinal cords of rats, in vitro. There results a pronounced swelling and fragmentation of the nerve fibers with demyelinization, destruction of glia nuclei and, apparently, granular disintegration of the nerve cells. This appears not to be due to the increased lipase content since after several days the lipase level fell while the toxic action persisted or became more pronounced. The authors succeeded in separating the toxic substance from the proteins of the blood.

They next studied changes produced in the nervous structures of dogs and rats which had been subjected to the above procedures and which were examined at intervals from ten days to six months after survival from the operation. In dogs they found, in general, spongy necrosis in the walls of the lateral ventricles with foci of edema in the white matter and extensive glia proliferation. Severe damage was usually found in the choroid plexes. This with the spongy degeneration of the ventricular walls suggested that the toxic substances were eliminated into the cerebrospinal fluid. The extent of damage decreased with increasing distance from the ventricles so that at more remote areas only nerve cell degeneration and glia proliferation were observed. Damage to the striate body was frequently seen.

In one dog and more consistently in the rats, another mode of elimination of toxic substances seemed to have occurred. In these, ventricular and choroid lesions were not observed but instead scattered foci of demyelinization around the blood vessels with overgrowth of fibrous glia, suggesting elimination through the vessel walls. Perivascular hemorrhages were observed early in this type of reaction.

Crandall and Weil point out the similarity of many of their pathologic findings to those observed in cases of Wilson's disease, the common features being particularly: spongy necrosis of nerve tissue, active proliferation of glia with the formation of large round or oval nuclei, diffuse nerve cell disease with predilection for the deep cortical layers and the striate body, with absence of active mesenchymal reaction. At the same time they point out that with the varied distribution and the character of the lesions, the same toxic factor can very readily produce a variety of combinations of pathologic lesions with a resultant distinct variability in symptomatology. "Such a point of view will prevent one from expecting stereotype encephalopathies in hepatic disease, or from assuming dogmatically one and the same etiology for all cases of hepatolenticular degeneration."

They conclude that experimental observations of hepatocerebral interrelationships such as those described will be more successful in demonstrating such relationships than clinical-pathologic observations since complicating factors such as arteriosclerosis and chronic focal infection which so often accompany hepatic and central nervous disease are thereby eliminated.

The observations of these investigators contribute further experimental corroboration of a phenomenon which has long been recognized clinically.

REFERENCE

Crandall, Latham A., and Weil, Arthur: Pathology of Central Nervous System in Diseases of the Liver; Experiments with Animals and with Human Material, Arch. Neurol. & Psych. 29: 1066, 1933.

—*W. T. V.*

OCT 2 _ 193

INDEX NUMBER

VOL. XVIII SEPTEMBER, 1933 No. 12

MEDICAL LIBRARY

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., EDITOR
808 Professional Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology
DENNIS E. JACKSON, M.D.
University of Cincinnati, Cincinnati

Physiology
J. J. R. MACLEOD, M.B.
Physiological Laboratory, Marischal College, Aberdeen, Scotland

Tuberculosis
GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

Clinical Pathology
W. C. MACCARTY, M.D.
Mayo Clinic, Rochester, Minn.

T. B. MAGATH, M.D.
Mayo Clinic, Rochester, Minn.

Biochemistry
VICTOR C. MYERS, PH.D.
Western Reserve University, Cleveland

Experimental Medicine
RUSSELL L. Haden, M.D.
Cleveland Clinic, Cleveland

Immunology
JOHN A. KOLMER, M.D.
Temple University, Philadelphia

ROBERT A. KILDUFFE, M.D.
Atlantic City, N. J.

Internal Medicine
GEORGE HERRMANN, M.D.
University of Texas, Galveston

Bacteriology
M. H. SOULE, Sc.D.
University of Michigan, Ann Arbor

Surgery
DEAN LEWIS, M.D.
Johns Hopkins University, Baltimore

PUBLISHED BY THE C. V. MOSBY COMPANY, 3523-25 PINE BLVD., ST. LOUIS

Entered at the Post Office at St. Louis, Mo., as Second Class Matter.

FOR OVER 31 YEARS

the cost of a $5,000.00—$25.00 accident policy has never exceeded $13.00 per year. No extra assessment has ever been called and one regular quarterly call was omitted in 1927.

The cost of each health policy has been raised from $17.00 to $20.00 per year to cover increase in claims.

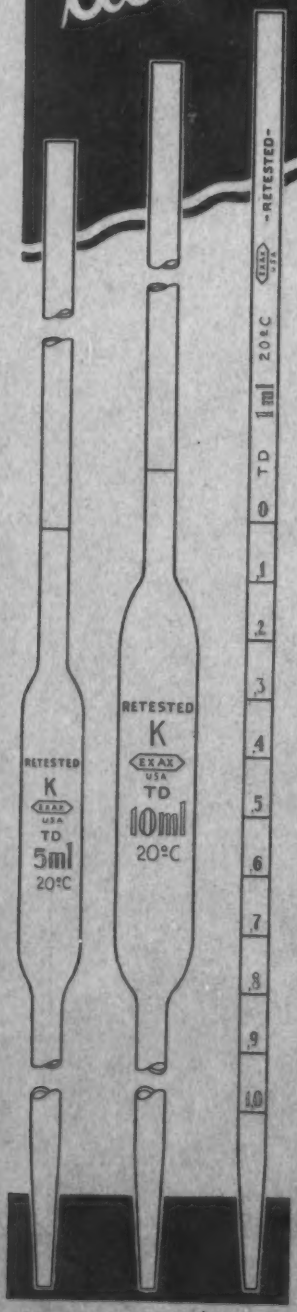
Over $1,000,000.00 assets to protect contracts.
Over $5,800,000.00 paid for claims.

Ethical physicians, surgeons and dentists not over 56 years of age, cordially invited to apply for membership.

PHYSICIANS CASUALTY ASS'N
OMAHA, NEBRASKA

PHYSICIANS HEALTH ASS'N
E. E. ELLIOTT, SEC'Y

Accuracy in BLUE LINE EXAX PIPETTES



KIMBLE Blue Line Exax Pipettes are made from extra heavy tubing of very uniform bore, automatic-machine-made, carefully selected to eliminate pieces with blisters, stones or streaks.

IMPORTANT FEATURES:

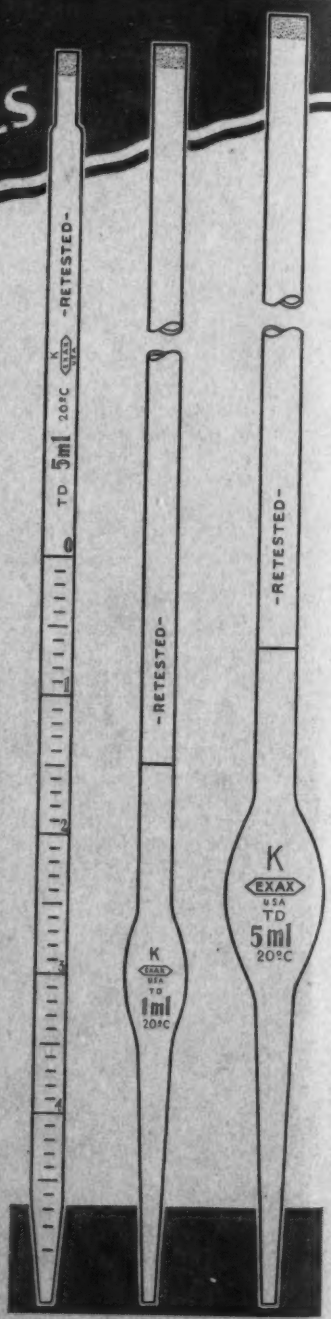
1. Designed to deliver contents accurately and at a proper speed.
2. Delivery ends are tapered gradually; tips are ground and slightly bevelled.
3. The tops are smoothly finished.
4. Volumetric and other pipettes have joints that are carefully made without constrictions.
5. Expertly calibrated at 20°C to deliver capacity by free outflow through tip. No drainage period allowed. Where the small amount remaining in the tip is to be added this is indicated by a frosted band at the top.
6. All calibration lines are deeply acid-etched and filled with a durable blue fused-in glass enamel.
7. An entirely new type of calibration lining for accurate and rapid reading. Short lines for intermediate divisions with encircling lines at the main graduation points.
8. Retested and retempered (strain-free).

VOLUMETRIC AND OSTWALD PIPETTES

Size	Tolerance	Size	Tolerance
1 *ml.*	±0.012 *ml.*	15 *ml.*	±0.06 *ml.*
2 *ml.*	.012 *ml.*	20 *ml.*	.06 *ml.*
3 *ml.*	.02 *ml.*	25 *ml.*	.06 *ml.*
4 *ml.*	.02 *ml.*	50 *ml.*	.10 *ml.*
5 *ml.*	.02 *ml.*	100 *ml.*	.16 *ml.*
10 *ml.*	.04 *ml.*	200 *ml.*	.20 *ml.*

MEASURING AND SEROLOGICAL PIPETTES

1/10 *ml.*	±0.005 *ml.*
2/10 *ml.*	.008 *ml.*
1 *ml.*	.02 *ml.*
2 *ml.*	.02 *ml.*
5 *ml.*	.04 *ml.*
10 *ml.*	.06 *ml.*
25 *ml.*	.10 *ml.*



STOCKED BY LEADING LABORATORY SUPPLY HOUSES THROUGHOUT THE UNITED STATES AND CANADA

KIMBLE GLASS COMPANY

VINELAND, NEW JERSEY.

NEW YORK · PHILADELPHIA · BOSTON · CHICAGO · DETROIT

SMALL-DOSE SALINE THERAPY

in its metabolic effect, distinguishes the marked results in treatment of overweight as achieved by

KRUSCHEN SALTS

The profession has been quick to discriminate between the accomplished therapeutic utility of this balanced formula in small-dose use as against the old-time argued hypothesis of depletion by catharsis.

The K. S. formula is a composite in therapeutic value of European spa waters the potability of which has long found medical service in weight reduction. Thousands of convincing clinical records received within the past few months from the American profession, attest the formula's high value.

Supplies for clinical tests are at every physician's command upon request.

E. GRIFFITHS HUGHES
INCORPORATED
ROCHESTER NEW YORK

Laboratory Colloid Mills

21527

Cut shows Eppenbach Laboratory Colloid Mill. This is made of best bronze, Rotor and Stator being of stainless steel. If desired, mills can be supplied of stainless steel at an extra charge.

SPECIAL FEATURES

a. Gap is fully adjusted and can be locked at any required setting.

b. Motor is for speeds up to 10,000 r.p.m. Material can be circulated for batch mixing, if desired.

c. Turbines produce immense pressure which forces the material through the grinding gap. These mills, therefore, are excellent for fine grinding as well as for homogenizing and emulsifying.

d. Mills can be quickly and easily taken apart for thorough cleaning.

e. Motor is Universal and connects to lamp socket.

USES

1. These mills are used for the production of all kinds of emulsions.
2. For thorough mixing, so as to combine a great variety of materials, such as carrots, berries and drugs to form a uniformly blended product.
3. The wet grinding of hard materials. For this use the sample is first brought down to about 20 mesh.

Write for Bulletin No. 472, which gives information in regard to the laboratory mills and also the industrial mills, where the requirements are larger and where continuous operation is an important factor

---

EIMER & AMEND

Est. 1851 Inc. 1897

Headquarters for Laboratory Apparatus and Chemical Reagents

Third Ave., 18th to 19th Street New York, N. Y.